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Treatment of Post-traumatic Simmonds' Disease with Methyl Testosterone Linguets

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IN 1942 Escamilla and Lisser (3) published a clinical study of Simmonds' disease including a statistical analysis of 595 cases, 101 of which were proved pathologically. A multiplicity of synonyms has been employed in reporting cases, especially the term "hypophyseal cachexia;" more recently, and now more frequently used, is the term "panhypopituitarism," introduced by Fuller Albright. It is reminiscent of the term, "hypopituitarismus totalis," used by Berblinger in 1934.

This severe endocrinopathy results from a more or less complete destruction of the pituitary gland, especially of the anterior lobe. This may be caused by a tumor, infarction with necrosis, infection, or it may be consequent to a traumatic injury.

The cardinal features of this malady are: (a) a marked loss of weight, often progressing to cachectic emaciation; (b) loss of sexual function—amenorrhea in the female, loss of libido and potentia in the male—and usually sterility in both sexes; (c) a low basal metabolic rate; (d) low excretion levels of follicle-stimulating hormone (6); (e) extremely low or absent excretion of urinary 17-ketosteroids (5). Asthenia is also a common symptom but does not seem to have as much diagnostic significance.

With the recognition of the primary responsibility of the pituitary gland for the widespread bodily damage produced by secondary derangements of the thyroid, adrenals, gonads, and, at times, the parathyroids and the insular portion of the pancreas, the goal of a well aimed endocrine replacement therapy would be, naturally, a supplementation of anterior pituitary failure by anterior pituitary extracts. However, the results obtained with available preparations, whether for stimulating growth of body tissues or for provoking more active gonadal function (not to mention adrenotropic or thyrotropic effects) have been inconsistent and dissappoint-

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ing. Indeed, occasional reports of beneficial results from their administration have almost always occurred in young unmarried females who, more likely than not, were suffering from anorexia nervosa, a psychic disorder, rather than from true Simmonds' disease.

As just stated, secondary disturbances of function in other incretory organs are a prominent feature of hypophyseal cachexia. Therefore, in view of the relative impotency of commercial anterior pituitary preparations, attempts have been made to influence the course of the disease by resorting to other hormones such as thyroid, adrenal cortical hormones, and the sex hormones. For the most part these efforts have failed also, except in the case of the male hormone, testosterone.

In 1942 Escamilla and Lisser (2) reported the case of a 20-year-old man who displayed the typical characteristics of Simmonds' disease caused by an intrasellar Rathke's pouch cyst. The cyst was drained and partially removed. Five months after the operation, no growth, appreciable gain in weight, or increase in sexual function had occurred, and the basal metabolic rate had remained at a low level, namely, minus 29 per cent. However vision had improved and general strength had increased somewhat. Subsequently, 19 months of testosterone therapy resulted in a gain of 10.6 kgm. (23¼ pounds) in weight, a growth of 4.5 cm. (1¾ inches), normal sexual function, enlargement of the genitalia, and improvement in secondary sexual characteristics. In addition to attending school, the patient was strong enough to work in the evenings as a pin-boy in a bowling alley.

Werner and West (7) reported subjective and objective improvement, nitrogen retention, weight gain, renewed strength and vigor, and restoration of libido in a girl of 20 and in a man of 31, who were believed to represent cases of Simmonds' disease consequent to intrasellar tumors, by daily oral doses of 100 mg. of methyl testosterone. Burke and Cantor (1) reported an idiopathic instance of Simmonds' disease in a 35-year-old emaciated male who had lost 25.5 kgm. (56 pounds); his pulse rate was 46 per min.; blood pressure, 94/66 mm. Hg.; and the basal metabolic rate, minus 34 per cent; he also had a secondary anemia, marked hypothermia, hypoglycemia, and hypochlorhydria. His complaints were fatigue, indigestion, nervousness, emotional storms, and a complete loss of libido. This patient was treated by parenteral injections of 25 mg. of testosterone propionate, given every four days, and 10 mg. of methyl, testosterone daily perorally. He gained 23.2 kgm. (51 pounds), and his pulse, blood pressure and basal metabolic rate became normal. His libido and potency were restored, and his wife became pregnant, whereas azoospermia had been found repeatedly prior to male hormone therapy.

Accordingly we welcomed the opportunity to utilize testosterone therapy again, this time in the form of linguets, in another male patient suffering from true Simmonds' disease, the pituitary failure in this case resulting from a severe motorcycle accident.

CASE REPORT[1]

R.M. was first seen at the University of California Hospital on November 9, 1936, at the age of 20 years. Previous to April 10, 1935, he had had no illness or injury of significance, but on that date he was involved in a motorcycle accident which resulted in severe, multiple fractures of the skull. For about seven weeks after the accident he was intermittently unconscious and irrational. After recovery from the acute phase of the injury there remained paralysis of the right side of the face, diminished vision and marked proptosis of the right eye, anosmia on the right side, and deafness of the right ear. A bruit could be heard over the entire skull, and a thrill and pulsation were palpable over the right eyeball. A diagnosis of arteriovenous aneurysm was made, and the right common carotid artery was ligated in November 1936.[2] This produced some improvement in the proptosis and slight increase in the visual acuity. In November, 1938,[2] an additional ligation of the right common carotid, right internal and external carotids and the superior thyroid arteries was done because of headaches and vomiting. This produced further improvement of the proptosis and relief from headaches and vomiting.

Immediately after the injury polydipsia, and polyuria to the extent of about four gallons daily, were noted. In the first two months there was a loss of 33 pounds of weight which was not regained.

[1] A brief preliminary report of this case, prior to testosterone therapy, was included in a paper on Simmonds' disease (3).

[2] Operations performed by Dr. Howard W. Fleming of San Francisco.

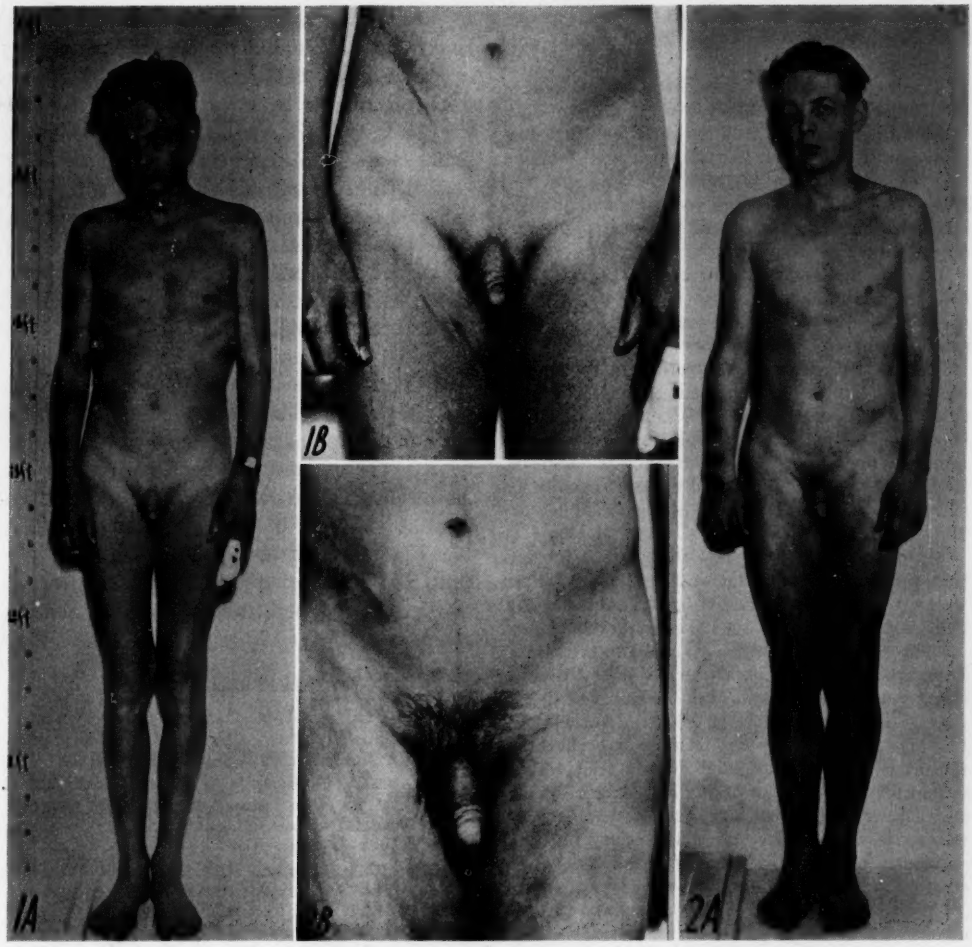

FIG. 1A. July 17, 1944. Weight, 64.2 kgm. (141.5 lbs.). Note asthenic habitus.
FIG. 1B. July 17, 1944. Note absence of pubic hair and relatively small penis and scrotum.

FIG. 2A. April 26, 1945. Weight, 73.7 kgm. (162.5 lbs.). Note gain of 9.5 kgm. (21 lbs.) from methyl testosterone linguet therapy. Note more alert attitude and erect carriage.
FIG. 2B. April 26, 1945. Note growth of pubic hair, penis and scrotum from methyl testosterone linguet therapy.

During the succeeding eighteen months there was progressive loss of facial and body hair, loss of libido and potentia, increasing fatigability, and the skin acquired a fawn color. There had also been a considerable decrease in the mental acuity with the concomitant development of phobias of dogs, heights, crowds, darkness, etc. At this time Dr. Lisser was called in consultation and he classified the endocrinopathy as "post-traumatic Simmonds' disease" or panhypopituitarism (involving loss of beard, axillary and pubic hair, and loss of libido and potentia, plus the changes in the nervous-emotional sphere characteristic of severe male hypogonadism). The blood pressure was 84/50 mm. Hg., and the basal metabolic rate minus 17 per cent. A glucose tolerance test consisting of 100 grams of glucose administered perorally gave the following values expressed in mg. per cent: fasting, 77; ½ hr., 135; 1 hr., 102; 2 hr., 58; 3 hr., 78; 4 hr., 53; 5 hr., 58.

During the years from 1936 to 1938 the patient received various pituitary extracts, which included a growth hormone, pituitary gonadotropin, and thyrotropic hormone, with little if any improvement.

During the years from 1938 to 1942 he received no special treatment, and no noteworthy changes occurred. In 1942 the patient began to have attacks

of pain in the chest and back and hematuria associated with nausea and vomiting. These attacks were never explained.

Injections of Armour's gonadotropin were started in October, 1943, by another physican,[3] in a dosage of 300 I.U. once a week, and the injections were continued until July, 1944. It was the patient's opinion that this medication increased his strength and stopped his pain, nausea and vomiting, and possibly increased the growth of general body hair; however, no gain in weight resulted.

On July 6, 1944, he reentered the University of California Hospital for study. During the hospital stay the basal metabolic rate was minus 28 per cent; serum cholesterol, 278 mg. per cent; insulin sensitivity test, according to the method of Fraser, Albright and Smith (4) was: fasting, 0.089; 15 min., 0.061; 30 min., 0.046; 45 min., 0.050; 60 min., 0.054; 90 min., 0.039; 120 min., 0.041; urinary 17-ketosteroids were 3.89 mg. per 24 hours (normal range, 6.0 to 21.0 mg. per 24 hours). Physical examination showed no special change from his earlier admissions (Figs. 1A and 1B).

On July 20, 1944, methyl testosterone linguets were prescribed in a dosage of 20 mg. daily for ten weeks, then 15 mg. daily for six weeks, and 5 mg. daily since November 9, 1944. The latter dose proved insufficient and was increased to 10 mg. daily on February 1, 1945. During the period of testosterone therapy a marked growth of pubic hair, beard, and general body hair occurred (Figs. 2A and 2B). Libido has returned to normal. Erections occur often, the patient has married, and marital relations are satisfactory. He is now working from 10 to 14 hours a day as manager of a large, wholesale, hardware warehouse with almost double the salary he could previously earn. His physical strength is now much greater, and there has been an increase of 9.5 kgm. (21 pounds) in weight (Fig. 2A). The patient states he feels just as well as before his accident except for his loss of vision in the right eye and loss of hearing in the right ear. In January, 1945, six months after testosterone therapy was instituted, the basal metabolic rate had risen from minus 28 per cent to plus 2 per cent, and the serum cholesterol had dropped from 278 mg. per cent to a normal value of 170 mg. per cent.

SUMMARY

Male hormone therapy, administered sublingually in the form of methyl testosterone linguets (5 mg. hard, compressed tablets[4]), has had a decidedly beneficial effect in a case of post-traumatic Simmonds' disease in a male patient aged 28 years. Prior efforts to mend his endocrine dysfunction by employing various pituitary preparations had failed to produce any convincing improvement.

As a consequence of the severe pituitary damage inflicted by trauma the patient had lost 15 kgm. (33 pounds) of weight, none of which had been regained; the sexual hair (beard, mustache, axillary and pubic) had dropped out completely; libido and potentia had vanished; and physical and mental inertia ensued and persisted. The basal metabolic rate was minus 28 per cent and the serum cholesterol, 278 mg. per cent.

In the course of nine months' treatment with methyl testosterone linguets the sexual hair reappeared, libido and erections returned to normal, physical strength and mental acuity was restored sufficiently to permit resumption of an arduous occupation, and the patient gained 9.5 kgm. (21 pounds). The basal metabolic rate rose from minus 28 per cent to plus 2 per cent, and the serum cholesterol dropped from 278 mg. per cent to a normal value of 170 mg. per cent.

[3] Dr. L. O. Kimberlin of San Francisco.

[4] These "Metandren Linguets" were furnished by Dr. Ernst Oppenheimer, Director of Medical Research, the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

REFERENCES

1. BURKE, G. and M. M. CANTOR. The treatment of Simmonds' disease with male sex hormones. *Canad. M. A. J. 52:* 275–277 (March, 1945).
2. ESCAMILLA, R. F. and H. LISSER. Testosterone therapy in male case of hypophyseal cachexia (Simmonds' disease). *Clinics 1:* 710–716 (Oct., 1942).
3. ESCAMILLA, R. F. and H. LISSER. Simmonds' disease: clinical study with review of literature; differentiation from anorexia nervosa by statistical analysis of 595 cases, 101 of which were proved pathologically. *J. Clin. Endocrinol. 2:* 65–96 (Feb., 1942).
4. FRASER, R. W., F. ALBRIGHT, and P. H. SMITH. Value of glucose tolerance test, insulin tolerance test, and glucose-insulin tolerance test in diagnosis of endocrinologic disorders of glucose metabolism. *J. Clin. Endocrinol. 1:* 297–306 (Apr., 1941).
5. FRASER, R. W., A. P. FORBES, F. ALBRIGHT, H. SULKOWITCH, and E. C. REIFENSTEIN, JR. Colorimetric assay of 17-ketosteroids in urine; survey of use of this test in endocrine investigation, diagnosis and therapy. *J. Clin. Endocrinol. 1:* 234–256 (March, 1941).
6. KLINEFELTER, JR., H. F., F. ALBRIGHT, and G. C. GRISWOLD. Experience with quantitative test for normal or decreased amounts of follicle stimulating hormone in urine in endocrinological diagnosis. *J. Clin. Endocrinol. 3:* 529–544 (Oct., 1943).
7. WERNER, S. C. and R. WEST. Nitrogen retention, creatinuria, and other effects of the treatment of Simmonds' disease with methyl testosterone. *J. Clin. Investigation 22:* 335–344 (May, 1943).

The Accumulation, Interpretation, and Presentation of Data Pertaining to Metabolic Balances, Notably Those of Calcium, Phosphorus, and Nitrogen[1,2]

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IN 1927, Bauer and Aub (12) described the ward routine and methods then employed in studies of inorganic salt metabolism on the Research Ward of the Massachusetts General Hospital. The present communication is a survey of the methods now employed by the authors. It will serve as a paper of reference as to the procedure used in studies already published (1, 4, 5, 7) and for others in progress; it is hoped, furthermore, that some of the methods, especially those concerned with the presentation of the data, may be adopted by others, thus establishing some uniformity in this field.

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[2] The authors are indebted to Esther Bloomberg, Dorothy F. Bryant, Evelyn Carroll, Lowell D. Cox, Eleanor F. Dempsey, Elizabeth C. Donaldson, Anne P. Forbes, Grace C. Griswold, Laurance W. Kinsell, and Priscilla White for technical assistance; and to Marion MacAulay, head nurse of Ward 4 since 1943, for her careful attention to the investigations.

Acknowledgment is made to the Schering Corporation, Bloomfield, New Jersey, for testosterone propionate (Oreton), for methyl testosterone (Oreton-M), and for estradiol benzoate (Progynon-B); to the Eli Lilly Company, Indianapolis, Indiana, for parathyroid extract; and to the Mead-Johnson Company, Evansville, Indiana, for casein hydrolysate (Amigen) preparations employed in these investigations.

[3] Dietitian, Ward 4.

I. ACCUMULATION OF DATA

A. *The metabolic unit*

The research unit (Ward 4) consists of 5 two-bed rooms under the charge of a graduate nurse and two assistants. There is a separate dietetic kitchen run by a full-time dietitian assisted by two student dietitians and a kitchen helper.

B. *General plan of experiments and control observations*

Most metabolic balance studies follow one of two plans: a) one in which the findings in one individual are compared with those in another, and b) one in which the findings in one individual on one regime are compared with the findings in the same individual on another regime. The second type of experiment is more satisfactory since any constant error, in all probability, will not modify the conclusions. It is of paramount importance that only one change in regime be made at one time. The investigator must be constantly on the alert to see that unintentional changes do not creep into the experiment; for example, changes in the amount of ultraviolet light in the atmosphere during the spring with its effect on calcium metabolism, or changes in the activity of the patient, *et*

cetera. If one anticipates such changes, one can often arrange to nullify them. Thus, if an experiment in calcium metabolism extends from winter months into the spring months it is advisable that vitamin D be administered throughout so that the changes in the quantity of ultraviolet light in the springtime will be relatively unappreciable; if the patient must be bedridden during part of the investigation, it may be advisable to have him confined to bed during the entire investigation. Whenever possible, and it usually should be possible, control regimes should both follow and precede each experimental regime. The shortest length of time that a regime must be continued to give accurate data before changing to another regime will depend on the problem being studied. If the fecal excretions are an important item (cf. calcium metabolic studies) each regime must last from two to three weeks. Fecal collections may fluctuate by 24 hours so that a collection for ten days may have a 10 per cent error from this source alone.

C. *Length of "metabolic periods"*

The authors have adopted a system whereby they collect stool specimens in metabolic periods of six days, and usually have at least three periods on each regime. One advantage of six is that it is divisable by six, three, two, and one, so that the urine periods can be one, two, three, or six days long, respectively depending on the circumstances. It is not necessary that urine and fecal periods be of the same length, and short urine periods are often desirable when rapid changes are anticipated (cf. nitrogen metabolic studies), and when it is desired to determine the sequence in which alterations appear. For example, Albright and Ellsworth (6) by employing metabolic periods of 8 hours duration were able to demonstrate that following the administration of parathyroid extract the urinary phosphorus excretion increased over 200 per cent during the very first period, and was maintained for five periods at approximately this elevated level before the urinary calcium excretion became increased. Furthermore, by repeating the experiment with metabolic periods of only one hour is duration, they showed that the excretion of urinary phosphorus increased in the very first hour after parathyroid hormone administration.

It is not practical to have fecal periods of less than six days because of the fluctuations; on the other hand, periods of more than six days involve specimens too large to be handled with ease. It is essential to have a minimum of two metabolic periods for each regime; otherwise one may miss an important trend due to some unforseen variable which may have crept into the experiment. If the results in these two periods check, all well and good; if not, one should have had a third period. A special word of warning must be inserted about a change from a diet high in a substance to a diet low in the same substance and *vice versa*. There will be a transitional period during the first few days before a new equilibrium is established. If the discrepancy of the transitional period is distributed throughout the entire regime during which the particular diet is employed, it may still be sufficiently great to lead to an erroneous interpretation of the effect of this diet on the balance (see Fig. 1). Therefore, one must disregard the transitional period in evaluating the level of the new equilibrium.

D. *Diet and medications*

1. *Factors governing the selection of a diet*

The type of diet selected depends on the information that is being looked for. If, for example, one is interested in the effect of estrogen on the calcium balance, one should give enough calcium to permit a positive balance, but not so much calcium that the expected deviation in balance will be lost in the error of the experiment. If, on the other hand, one is studying the rate of destruction of bone by a pathologic process, the lower the calcium intake the better. It was the recognition of this important principle which first led Dr. Joseph C. Aub to employ the "low calcium diet" in studies on calcium metabolism (12). There will be other considerations: the acid-base balance of the ash of the diet if the studies concern calcium metabolism, the nitrogen content, the caloric requirements, seeing to it

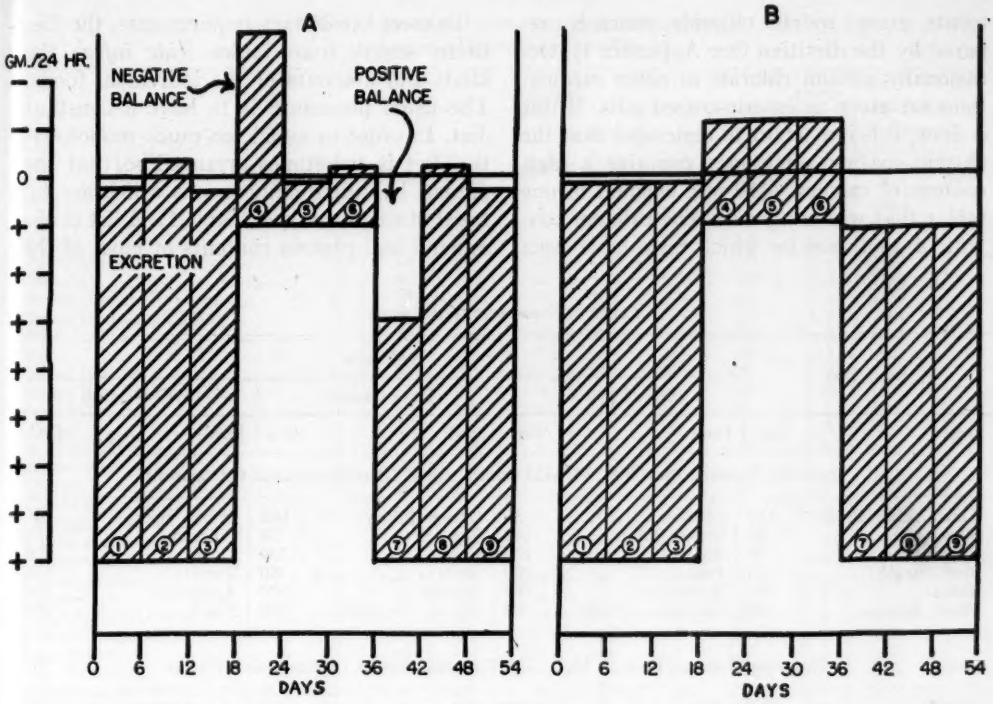

FIG. 1. *Diagram to illustrate the effect of alterations in the content of a dietary constituent on the excretion of that constituent, and to show the error in interpretation if the transitional periods are not charted separately*

This diagram is constructed from hypothetical data according to the method for presentation of metabolic data advocated by the authors. It is constructed as follows: a) the scale for intake and balance in gm./24 hr. is given as the ordinate; b) the scale for time (in this case days) is given as the abscissa; c) the horizontal line at 0 of the ordinate is the base line to which intake and balance refer; d) the intake is plotted as an area from the base line toward the bottom of the diagram; e) the excretion is plotted as a hatched area from the bottom of the intake toward the top of the diagram. If the excretion does not reach the base line, a white area is left between the excretion line and the base line; this represents a positive balance. If the excretion reaches the base line, the balance is in equilibrium. If the excretion exceeds the base line, a hatched area is left above the base line; this represents a negative balance. The data are plotted in amounts per 24 hours although the measurements are made on pools of excreta covering the metabolic periods (in this diagram six-day periods, numbered in circles 1 to 9). For simplicity in this particular diagram the excreta are not divided into fecal and urinary amounts; when this division is made (see Figures 5, 8 and 10) the fecal excretions are charted beneath, and the urinary excretions above the fecal excretions.

In this chart there are two divisions (A and B) in which the same data are plotted in two different ways. In both, periods 1, 2, 3, 7, 8, and 9 show a high intake, while periods 4, 5, and 6 show a low intake. In A the excretion is charted separately for each period; in B the excretion is charted as averages of three periods at a time. It will be seen in A that when the intake level is reduced there is a lag in the fall in excretion in period 4 so that the balance in this period is negative; similarly it will be seen that when the intake level is increased there is a lag in the rise in excretion in period 7 so that the balance in this period is positive. Furthermore, the lag in period 4 equals the lag in period 7. However, it will be noted that the balance is approximately in equilibrium in periods 5 and 6, and in periods 8 and 9. Hence, the changes in intake level produced no permanent alterations in the balance. When the same data are plotted as in B, it will be seen that the balance was negative in periods 4, 5, and 6, and positive in periods 7, 8, and 9. Hence, in B the changes in the intake level erroneously appear to have produced permanent alterations in the balance.

For further discussion, see text.

that the diet is not insufficient in some constituent (phosphorus, potassium, vitamins, essential amino acids, etc.).

To make the calcium intake as accurate as possible it is preferable to keep the calcium content of the diet itself low and to supplement it with the desired amount of calcium in the form of some compound such as calcium gluconate (C. P. grade). The calcium to be added each day is weighed out in the laboratory and divided by the nurse into approximately equal doses which are given at intervals throughout the day. The same procedure is followed with other mineral supple-

ments, except sodium chloride, which is prepared by the dietitian (see Appendix 1). Occasionally sodium chloride or other medications are given as enteric-coated pills. If this is done, it is important to remember that the enteric coating sometimes contains a high content of calcium (110 mg. calcium in one tablet that was analyzed). The calcium content of toothpowder which may have been swallowed should not be overlooked (Pebecco Brand toothpowder was found to contain 35.9 grams of calcium per 100 grams of powder).

If one desires to take into account the acid-base content of the ash of the diet, it is usually preferable, rather than to ask the dietitian to select certain foods to meet this end, to weigh out and add to the intake acid or basic salts (*e.g.*, NaH_2PO_4, NH_4Cl, Na citrate, or Na gluconate) sufficient to produce the desired shift in the acid-base content. Furthermore, in changing from one diet to another, the acid-base content of the ash of the diet or the amount of any or all of the electrolytes can be kept constant by a similar procedure.

To meet the dietary requirements, the dietitian selects from tables (*vide infra*) the kinds and amounts of the individual foods. The usual procedure is to have a constant diet. In order to avoid too much monotony, the diet is sometimes arranged so that the patient has three daily menus which are rotated in succession (see Table 1). A list of the mineral and protein contents of most of the foods employed in the studies of the authors is given in Table 2. These values have been obtained in part by analyses (12), and in part from the literature (15, 45, 46, 47, 51, 54).

TABLE 1. SAMPLE ROTATING DIETS

Basic Diet		Additions					
		Days 1 and 4		Days 2 and 5		Days 3 and 6	
Food	Gm.	Food	Gm.	Food	Gm.	Food	Gm.
Three-day Rotating Diet: F.F., #156453, female, age 42, Post Menopausal Osteoporosis							
Low Calcium Bread	130	Chicken	56	Orange Juice	100	Orange Juice	105
Rice	20	Orange Juice	110	Egg	50	Chicken	56
Spaghetti	20	Tomato	100	Corn	100	Carrot	100
Beef (Steak)	80	Peas	100	Beets	80	Tomato	100
Potato	65	Applesauce	100	Banana	100	Applesauce	100
Heavy Cream	20	Grapefruit (Fresh)	100	Orange (Sections)	100	Peach	100
Three-day Rotating Diet: C. McC., #384774, male, age 26, Osteoporosis of Disuse							
Orange	270	Peas	100	Corn	100	Beans (Wax)	100
Low Calcium Bread	150	Tomato	100	Beans (Green)	100	Beets	100
Beef (Steak)	150	Peach	200	Pineapple	200	Apricots	200
Onion	15	Pear	200	Applesauce	200	Grapefruit (Fresh)	200
Catsup	20						
Jelly	30						
Potato	100						
Turkey	70						
Egg	150						
Rice	40						
Spaghetti	40						

With all of this discussion of calories, electrolytes, etc. the art as opposed to the science of dietetics must not be lost sight of (11, 30). The diet must be sufficiently attractive to the patient so that he will eat it for as long a period as desired; this often may extend into months. A few days spent at the outset in rearranging the diet to meet the patient's desires may save trouble later on.

2. *Determination of the contents of a diet*

The usual methods for determining the

TABLE 2. MINERAL AND PROTEIN CONTENT OF FOODS
(Expressed in Amounts per 100 Grams)

Type of Food	milligrams							grams		
	Calcium	Magnesium	Potassium	Sodium	Phosphorus	Chloride	Sulfur	Protein	Reference to Source of Data	Footnote
Apple	7	8	127	11	12	5	6	0.2[4]	(46)	[5]
Apricots (Fresh)	13	10	248	38	24	2	10	0.6[4]	(46)	
Banana	8	30	391	31	28	129	12	1.2	(46)	
Beans (Lima) (Fresh)	28	70	613	88	133	9	57	7.5	(45)	
Beans (String and Wax)	55	26	223	18	50	38	35	2.4	(46)	
Beef	12	25	361	90	230	81	244	21.3	(46)	
Beets	28	26	355	97	42	58	18	2·3	(46)	
Bread (Graham)	50	50	291	394	218	607	150	8·9	(45)	
Bread (Low Calcium)	11	23	108	394	82	607	105	8.0	(12)	
Bread (White)	31	23	108	394	97	607	105	9.2	(46)	
Butter	15	1	14	788	17	1212	10	1.0	(45)	
Butter (Salt-Free)	15	1	14	6	17	13	10	1.0	(45)	[6]
Butter Fat	0	0	0	0	0	0	0	0		[7]
Carrots	45	20	308	77	41	39	22	1.2	(46)	
Casein Hydrolysate (Amigen) Powder	408	—	—	—	835	—	—	73.0		[8]
Casein Hydrolysate (Amigen) Solution	191–236	—	164–174	—	774–833	—	—	81.7–76.8		[8]
Cheese (Yellow)	930	37	89	606	701	880	263	24.4	(46)	
Chicken	18	36	460	94	161	54	314	21.5	(12)	
Corn (Sweet) (Fresh)	6	33	113	40	103	14	46	2.8	(46)	
Cream (Average)	90	10	130	30	80	80	30	2.5	(46)	
Egg (Whole)	63	10	138	140	224	117	204	13.4	(46)	
Egg (White)	13	11	155	168	15	159	214	12.3	(46)	[9]
Grapefruit (Fresh)	21	9	161	4	20	5	10	0.5	(46)	
Lemon (Juice)	22	9	123	6	11	6	9	0.9	(46)	
Lettuce (Head)	17	12	332	20	40	79	14	1.2	(46)	
Milk (Whole)	118	12	143	51	93	106	34	3.3	(46)	
Milk (Fat-Free)	122	12	149	52	96	110	35	3.4	(45)	
Oatmeal	65	122	395	68	387	49	199	16.1	(46)	
Onion	41	17	185	18	47	24	75	1.6	(46)	
Orange (Sections)	24	11	181	12	18	5	10	0.8	(46)	
Orange (Juice)	24	11	181	12	18	5	10	0.0	(46)	
Peaches (Fresh)	10	10	214	22	19	4	9	0.4[4]	(46)	[10]
Pears (Fresh)	15	10	132	10	18	5	7	0.2[4]	(46)	
Peas (Fresh)	23	34	285	13	127	24	63	3.6	(46)	
Pineapple (Fresh)	8	10	270	16	11	51	9	0.4[4]	(46)	
Potatoes	13	28	456	22	53	40	35	2.0	(46)	
Prunes (Dried)	58	55	1030	69	85	17	37	2.1	(46)	
Rice (White)	11	33	70	25	99	40	117	8.0	(46)	
Spaghetti	22	37	130	8	144	73	172	13.4	(46)	[11]
Squash (Winter)	19	11	320	4	28	6	89	1.4	(46)	
Tomatoes (Including Seeds)	11	13	267	12	29	29	14	1.2	(46)	
Tomatoes (Juice)	7	10	267	12	15	54	5	2.0[4]	(46)	
Turkey (Cooked Light Meat)	23	28	367	130	320	123	234	34.5	(47)	[12]
Walnuts (English)	89	134	332	(26)	358	40	172	18.4	(46)	[13]

[4] Protein in figures for canned fruits from Bowes and Church (15).
[5] Mineral figures are those of Sherman (46) for applesauce.
[6] Sodium and chloride calculated from amount of milk containing 15 gm. of calcium.
[7] Mineral and protein content assumed to be negligible.
[8] Assayed in our laboratory. The range of constituents in casein hydrolysate solutions is based on analyses of 4 batches of 10% Amigen solution.
[9] Protein figure from Waller (54).
[10] Sodium and chloride figures from Sherman (45).
[11] Calculated from macaroni.
[12] Protein figure from Taylor (51).
[13] Sodium figure calculated from chloride on assumption that it is present as sodium chloride.

contents of a diet have been discussed by Bassett and Van Alstine (11). These methods involve either: a) the use of average values for the contents of foodstuffs obtained from tables compiled by other investigators (11, 15 40, 45, 46, 47, 51, 54). or b) the use of values

obtained by the investigator through his own analyses.

a. *Determination of the contents of a diet by tables*

The use of tables immediately recommends itself. However, before this procedure can be adopted, it is necessary to establish the validity of the tables by actual analysis of foods or diets representative of those to be used in the proposed metabolic studies. The results of such a comparison are given in Table 3, in which the contents of minerals and of protein as determined by calculation from the values of Table 2 are compared with those obtained by analysis. In general, the analyzed content is less than the calculated content. Thus, the analyzed values for calcium varied from 49.5 to 91.1 per cent of the calculated with an average of 69.9 per cent. This discouraging discrepancy is in agreement with that obtained by Gutman and Low (31), who compared analyzed values for low calcium diets with values calculated from the tables of Sherman (46) and found the average of the former to be only 75.3 per cent of the latter. A similar discrepancy was obtained by Bassett and Van Alstine (11), who compared the mean analyzed value for 10 identical low calcium diets (0.15 gm./24 hr.) with the value calculated from earlier tables of Sherman (44) and found the former to be only 67.4 per cent of the latter. The cause of this discrepancy has not been investigated. To be sure,

TABLE 3. A COMPARISON OF THE ANALYZED AND THE CALCULATED CALCIUM, PHOSPHORUS AND NITROGEN CONTENT OF DIFFERENT DIETS

Patient	Unit Number	Date	Calcium			Phosphorus			Nitrogen		
			Analyzed mg./24 hr.	Calculated mg./24 hr.	Recovery per cent	Analyzed mg./24 hr.	Calculated mg./24 hr.	Recovery per cent	Analyzed gm./24 hr.	Calculated gm./24 hr.	Recovery per cent
A.H.	3276	Apr. /39	57	70	81.4	475	475	100.0	—	8.12	—
M.A.	168199	10/ 2/43	73	96	76.0	595	582	102.2	8.10	8.78	92.2
E.S.	360207	Aug. /42	57	100	57.0	566	805	70.4	9.90	10.53	94.0
M.A.	168199	Dec. /41	61	104	58.6	573	673	85.3	9.37	8.78	106.7
M.H.	278511	Sept. /42	54	109	49.5	569	625	91.1	9.09	8.13	111.7
		Oct. /41	71	109	65.1	584	671	87.0	9.31	8.13	114.5
B.V.	74372	2/16/39	79	126	62.7	336	482	69.7	—	6.88	—
		2/18/39	82	137	59.9	323	489	66.0	—	6.77	—
		2/19/39	83	142	58.5	345	439	78.5	—	7.06	—
F.F.	156453	Nov. /38	102	154	66.2	603	688	87.7	—	8.14	—
			112	164	68.3	600	600	100.0	—	7.76	—
			114	172	66.3	628	703	89.4	—	7.49	—
M.H.	278511	Sept. /42	93	182	51.1	864	1234	70.0	1.74	1.63	106.2
F.F.	156453	Nov. /38	161	187	86.0	604	701	86.2	—	7.33	—
A.R.	29358	May /39	131	188	69.7	668	742	90.0	—	8.56	—
F.F.	156453	Nov. /38	143	194	73.7	596	632	94.3	—	7.97	—
I.G.	350260	10/10/42	119	198	60.1	587	691	84.9	13.37	14.05	95.3
H.D.	382395	Feb. /43	177	202	87.5	760	927	82.0	11.11	11.30	98.3
R.B.	3397	Mar. /43	183	213	85.9	804	818	98.8	13.20	11.88	111.6
K.McK.	385585	11/19/43	198	221	89.5	770	909	84.7	9.46	10.44	90.7
S.G.	385496	2/25/43	137	224	61.2	606	707	85.7	5.35	5.83	91.8
R.H.	401611	May /43	138	247	55.8	636	830	76.6	13.07	13.43	97.3
C.O'R.	380940	10/ 5/43	212	320	66.3	1125	1286	87.4	19.40	18.43	105.3
H.B.	425355	12/ 5/43	259	337	76.8	1106	1298	85.2	13.41	14.99	89.4
		11/10/43	274	350	78.3	1113	1298	85.7	14.89	14.97	99.0
C.McC.	384774	3/ 2/43	275	359	76.6	1173	1448	81.0	16.05	16.99	94.4
C.O'R.	380940	Apr. /43	333	471	70.7	1503	1797	83.6	20.79	23.12	90.0
F.D.	136431	Oct. /43	744	815	91.1	1015	1231	82.1	9.25	9.65	95.8
H.LaB.	369099	9/15/42	—	1326	—	—	2115	—	18.08	19.67	91.8
	Average				69.9			85.4			98.7
	Range				49.5 to 91.1			66.0 to 102.2			89.4 to 114.5

most of the diets in Table 3 are low calcium diets (analyzed content under 0.15 gm./24 hr.), so that a small actual error makes a large percentage error. For example, in Table 3 the 18 diets with an analyzed calcium content of less than 150 mg./24 hr. deviated from their calculated content by an average of a mere 56 mg./24 hr., which, none the less, was 37.1 per cent of the calculated.

The analyzed phosphorus figures in Table 3, although better than the calcium, are still far out of line. Thus, the analyzed values varied from 66.0 to 102.2 per cent of the calculated with an average of 85.3 per cent. The analyzed nitrogen values varied from 89.4 to 114.5 per cent of the calculated with an average of 98.7 per cent. Bassett and Van Alstine (11) in their analyses of 10 identical low calcium diets obtained a mean phosphorus value that was 94.6 per cent and a mean nitrogen value that was 115 per cent of the corresponding values calculated from the earlier tables of Sherman (44). For a similar analysis of other minerals see Bassett and Van Alstine (11).

Because of the errors introduced by computation of intake from tables, great caution must be exercised in using values so obtained. For determining absolute balance values tables are of little value; for comparing one regime with another they still may be of use. Obviously, if the intake has a 10 per cent error one cannot decide whether the patient is in positive or negative balance unless the balance is greater than 10 per cent of the intake. However, when the patient is on a constant intake the intake error should be constant, and an otherwise significant deviation from the control values resulting from the introduction of the variable being studied should be valid.

Even where the exact intake is to be determined by analysis, tables are of use to enable the dietitian to approximate the dietary requirements at the onset of a study. The authors employ Table 2 chiefly for this purpose.

b. *Determination of the contents of a diet by analysis*

For accurate metabolic studies it is usually necessary to analyze the diet. Three procedures may be followed: (a) each foodstuff may be analyzed at the start and the values obtained used throughout; (b) one or more sample diets may be analyzed *in toto* and the results employed throughout; or (c) aliquots of the diet as served each day may be pooled and analyzed for each period. The disadvantage of (a) and (b) is that foodstuffs fluctuate in their composition from day to day (*vide infra*); the disadvantage of (c) is its laboriousness. In most instances we have adopted procedure (b), because (c) involves too much labor for the slight additional accuracy obtained.

It is recognized that the mineral and protein contents of the same foodstuffs fluctuate from time to time, depending upon such factors as soil, climate, method of preservation, *et cetera*. These fluctuations in the content of the diets undoubtedly can be decreased by a careful selection of the type of food employed, and by the purchase of a large amount of each commodity from one stock source at the beginning of each experiment (11, 31, 35). Our data pertinent to this point are given in Table 4. The same diet was compared two times in nine instances and three times in one instance after periods of time that varied from two weeks to over two years. There were average deviations of approximately seven per cent (12 mg.), five per cent (37 mg.), and 11 per cent (1.3 gm.) for calcium, phosphorus, and nitrogen, respectively. There appeared to be no correlation between the degree of variation in these three components. If one allows for the fact that the actual calcium intake was small in the majority of these diets, the per cent deviation of calcium is not unduly high; the same holds for the phosphorus. However, in several instances the nitrogen values are way out of line. It is desirable, therefore, that analyses for nitrogen be made on at least two diets, and on a third if the first two are not in agreement. Pertinent to this discussion are the figures of Bassett and Van Alstine (11). In a series of 10 "identical" low calcium diets they obtained the following coefficients of variation: nitrogen, 2.3; sodium, 2.3; potassium, 0.8; calcium, 8.8; magnesium, 2.8; chlorine, 6.1; phosphorus, 3.0; and iron, 8.6 per cent; in a

series of 11 "identical" high calcium diets the coefficients of variation were: nitrogen, 2.9; sodium, 3.2; potassium, 3.4; calcium, 7.5; magnesium, 2.6; chlorine, 3.7; phosphorus, 5.1; and iron, 7.6 per cent.

Variations that arise in the process of analysis may be due to errors in sampling in the diet kitchen, or to errors introduced by the analytical methods and manipulations in the laboratory. The former are not readily susceptible to statistical measurement. However, the authors agree with Bassett and Van Alstine (11) that gross inaccuracy in sampling is unlikely. The latter investigators have evaluated the technical errors of the laboratory by making repeated analysis from the same high calcium food sample. They obtained the following coefficients of variation: sodium, 1.36; potassium, 1.65; calcium, 0.96; magnesium, 2.37; chlorine, 2.66; and phosphorus, 1.61 per cent. These variations are smaller than those found in different lots of the same foodstuffs from time to time (*vide supra*). As Bassett and Van Alstine point out, the fluctuations in the analyzed values of these foodstuffs must represent fortuitous variations in the concentrations of the various minerals and protein in the same food from

TABLE 4. A COMPARISON OF THE ANALYZED CALCIUM, PHOSPHORUS, AND NITROGEN CONTENT OF THE SAME DIET AT DIFFERENT TIMES

Patient	Unit Number	Date	Calcium			Phosphorus			Nitrogen		
			Analyzed[14] mg./24 hr.	Deviation		Analyzed[14] mg./24 hr.	Deviation		Analyzed[14] gm./24 hr.	Deviation	
				mg.	%		mg.	%		gm.	%
E.S.	360207	8/ 1/43 2/ 1/44	57 65	8	12.8	566 633	67	10.6	9.90 8.67	1.23	14.2
M.H.	278511	Oct. /41 2/ 2/44	71 64	7	10.9	584 611	27	4.5	9.31 8.40	0.91	10.8
I.G.	350260	10/10/42 10/12/42 10/26/42	119 110 104	15[15]	14.4[15]	587 570 582	17[15]	2.9[15]	13.37 12.86 12.65	0.72[15]	5.7[15]
K.McK.	385585	11/19/43 2/ 1/44	198 198	0	0.0	770 854	84	9.9	9.46 10.97	1.51	13.8
S.G.	385496	2/25/43 2/ 3/44	137 137	0	0.0	606 634	28	4.5	5.35 5.69	0.34	6.0
R.H.	401611	5/27/43 2/ 2/44	138 148	10	6.8	636 642	6	0.9	13.07 10.81	2.26	20.9
I.G.	350260	Feb. /43 Jan. /44	139 166	27	16.3	1036 1089	53	4.9	16.22 17.26	1.04	6.1
C.O'R.	380940	10/ 5/43 2/ 3/44	212 209	3	1.4	1125 1135	10	0.9	19.40 16.74	2.66	15.9
H.B.	369099	12/ 5/43 1/ 4/44	259 260	1	0.4	1106 1103	3	0.3	13.41 14.99	1.58	10.6
F.D.	136431	Oct. /43 2/ 1/44	744 696	48	6.9	1015 1089	74	6.8	9.25 9.71	0.46	4.7
Average				11.9	6.99		36.9	4.62		1.27	10.87
Range				0 to 48	0.0 to 16.3		3 to 84	0.3 to 10.6		0.34 to 2.66	4.7 to 20.9

Calculated values for these diets are found in Table 3.
[14] Average of two or more aliquots.
[15] Maximum deviation.

time to time. It would be more accurate, therefore, to sample and analyze the food given in each metabolic period.

E. *Fluid intake*

It may be desirable to keep the fluid intake constant. It should be remembered, however, that there are varying amounts of fluid lost in the perspiration, in the feces, etc., and that in the interests of constancy it may be better to allow the patient to drink water as desired. Within reasonable limits, the amount of the fluid intake has very little effect on most metabolic balances. If a constant fluid intake is decided upon, the dietitian calculates the fluid taken as such in the diet, and the nurse makes up the difference with water (see Appendix 1). The water content of solid foods and water of oxidation are usually ignored in calculating the fluid intake. The mineral content of Boston water can be disregarded in most experiments (calcium, 4 mg., and phosphorus, 6 mg. per 1000 cc.); in some localities the mineral content of tap water would have to be considered, or distilled water would have to be used (in one specimen of tap water the calcium content was 30 mg. per 1000 cc.).

F. *Collection of specimens*

Urine collections are begun at six o'clock in the morning, and are collected in 24 hour amounts which are kept in the ice-box without preservative. Unless nitrogen determinations are to be done, stool specimens are collected in a cellulose film bag[16] which is kept in a covered jar. When nitrogen is to be determined, the individual stool specimens are placed in separate covered glass jars and stored on ice. The stool periods are marked with 0.6 gm. carmine alum lake by mouth given at six p.m. on the last day of the period; the first carmine-colored stool on the next day is included in the period that is being terminated. If necessary, and if absorption from the gastro-intestinal tract is not a primary factor in the study, 0.2 gm. cascara (the calcium and phosphorus content of which is so small that it is usually ignored) is given with the carmine. If an enema must be given, a measured quantity (200 to 600 cc.) of distilled water is used. Preferably one should either use cascara or an enema after every period or not at all. Vomitus may be analyzed as such and considered to be rejected intake, or added to the stool specimens and analyzed as additional output depending upon the circumstances. The specimen of vomitus is prepared for analysis in the same manner as is the diet (*vide infra*).

G. *Preparation of specimens for analysis*

1. *Diets*[17]

A suitable aliquot (*circa* 50%) of the diet is transferred to an evaporating dish. Butter fat, NaCl, occasionally sugar, and milk (if in large amounts) are usually excluded from the aliquot and analyzed separately. The evaporating dish is placed in a steam-drying-oven until the material can be crumbled readily. This usually requires about seven days. The aliquot is then ground with a hand mechanical grinder, and further pulverized, if necessary, in a mortar with a pestle until it forms a fine homogeneous powder; the total weight of this is then obtained. For the nitrogen determination, a suitable aliquot (usually 0.5 gm.) of this powder is weighed dry, and then digested in the usual manner. For mineral determinations, a suitable aliquot (usually 25 grams) is weighed dry, and then placed in an evaporating dish in a muffle-oven for about 16 hours at a temperature of 500 to 600 degrees Centigrade. This material is then extracted with 10 per cent hydrochloric acid at least three times or until the residue is negligible. The extract is then fil-

[16] A sufficiently large sheet of cellulose film is pushed into the stool jar so that a pocket is formed into which the stool specimens are scraped. The ends of this sheet of film are allowed to extend over the lip and outside the cover of the jar. When the period is completed, the ends of the film are gathered up and twisted to form a bag. "Cellophane" Brand, No. 300, moixture-proof, transparent cellulose film manufactured by the Dupont Company is used.

[17] After the data given in Tables 3 and 4 were compiled, the method of preparing diets for analysis was changed. Diets are now prepared by a "wet method" similar to that used for stool specimens (*vide infra*). A suitable aliquot of the diet is thoroughly mixed in an electric mixer (Waring Blender) with distilled water, and made up to a suitable known volume. Aliquots for nitrogen and mineral determinations are taken as with stool specimens. This change has improved the accuracy of the dietary analyses and the ease in handling the diet aliquot.

tered through Whatman's #44 ashless filter paper; if there is a residue, the filter paper is then ashed in the muffle-oven for 16 hours, and finally extracted three times with 10 per cent hydrochloric acid. The combined hydrochloric acid extracts are made up to a suitable known volume (usually 100 cc.) in a volumetric flask. Suitable aliquots of this are then taken for the determinations.

2. *Stools*

Stool specimens are collected by two methods: the "dry method" if nitrogen determinations are not going to be done, and the "wet method" if nitrogen determinations are to be done (*vide supra*). In the "dry method" the cellulose film bag (*vide supra*) containing the stool is placed in a large evaporating dish and dried in the steam-drying-oven; this usually takes two days. The surplus cellulose film is then burned off the specimen, and the evaporating dish is put in the muffle-oven at 500 to 600 degrees Centigrade for about 16 hours. Thereafter, the extraction with hydrochloric acid is carried out in the same manner as it is in the preparation of a diet for analysis. The combined acid extracts are made up in a volumetric flask to a known volume (usually 500 cc. for a six-day period). This concentrated extract has been stored for months successfully. With distilled water suitable dilutions of this solution (usually 1:10) are made on the basis of the estimated mineral content of the specimen. Aliquots of the dilutions are taken for the determinations.

Stool specimens collected by the "wet method" are thoroughly mixed in an electric mixer (Waring Blender) with distilled water and made up to a suitable known volume for pipetting. For nitrogen determinations, 10 cc. aliquots are pipetted directly into the digestion flask and digested using 20 cc. of concentrated sulfuric acid instead of the 10 cc. usually employed for urine specimens. For mineral determinations, a suitable aliquot of the mixed and diluted specimen (usually 250 cc. of a four-liter volume of a six-day stool specimen) is dried in an evaporating dish in the steam-drying-oven. This requires 24 hours. The aliquot is then placed in the muffle-oven at from 500 to 600 degrees Centigrade for about 16 hours. Thereafter, the extraction with hydrochloric acid is carried out in the same manner as it is in the "dry method."

3. *Urines*

The 24-hour urine specimens are kept in the refrigerator each day during the period of collection. As soon as the specimen is completed, it is measured, and aliquots removed for creatine and creatinine determinations or for biological assays if these are being done. An aliquot of the remainder of the specimen is then acidified with 0.5 cc. of concentrated hydrochloric acid per 100 cc. and stored at room temperature. At the end of the metabolic period, the aliquots of the daily urine specimens are mixed together; from this mixture aliquots are taken for nitrogen and mineral determinations.

H. *Methods of analysis*

The methods of analysis used in the laboratory are, in general, standard techniques. Urine and stool specimens are analyzed for calcium by the method of Fiske and Logan (24); phosphorus by the method of Fiske and Subarrow (25); nitrogen by the Kjeldahl method of Folin (27); and potassium by the method of Fiske and Litarazek (23). The sulfur content of the urine is determined by the method of Fiske (22); the creatine and creatinine content of the urine by the method of Folin (26). The 17-ketosteroid excretion in the urine is determined by the modification of the Zimmermann reaction (58) introduced by Callow, Callow and Emmens (17) and subsequently further modified by Fraser, Forbes, Albright, Sulkowitch and Reifenstein (29). As far as possible, an effort is made to extract the urine with carbon tetrachloride on the same day that the specimens forming a metabolic period of from three to six days are mixed together (*vide supra*).

The following methods have been employed for serum: calcium, Fiske and Logan (24); phosphorus, Fiske and Subarrow (25); alkaline phosphatase, Bodansky (14); potassium, Fiske and Litarazek (23); sugar, Folin (28); chloride, Wilson and Ball (55); sodium, Butler and Tuthill (16); protein, Lowry and Hunter (38); and carbon dioxide, Van Slyke

(52). The basal metabolic rate is determined by the closed circuit method of Benedict and Roth (41).

I. *Weighing of patients*

Patients are weighed daily under standard conditions: after voiding, but before breakfast; they should be nude. Patients confined to bed are lifted onto a long table counterbalanced on a scale. Dressings or casts are weighed on removal.

II. INTERPRETATION OF DATA

A. *Detection of gross errors*

Before one attempts to evaluate the significance of one's data it is wise to make a few simple calculations to see whether the results are reasonable. Some trivial error can easily creep in, such as an error in a decimal point; in this laboratory all such errors go under the generic term "decimalitis."

To accomplish the above purpose it is most helpful to derive certain data from the measured data. Thus, in an experiment involving calcium, phosphorus and nitrogen, one can compare the measured phosphorus balance with the "theoretical phosphorus balance" to be expected from the determined calcium and nitrogen balances (*vide infra*); in an experiment involving changes in body protoplasm one can see whether the sulfur, potassium, phosphorus, nitrogen, and weight alterations have fluctuated in relation to one another as they should if there had been changes in body protophasm (*vide infra*). Any discrepancy from the expected findings should be carefully checked before it is accepted. Factors involved in these derived data will be discussed below.

B. *What constitutes significant data?*

It is beyond the scope of this presentation to discuss the mathematical significance of data. However, there are a few points concerning the significance of data that should be stressed.

It is desirable that one decide first whether observed fluctuations in the accumulated data are significant before one attempts their interpretation. For example, since dietary intake in most instances is accurate within only 10 per cent (*vide supra*), any fluctuation in the balance which is not more than that produced by a 10 per cent change in the intake is of doubtful significance.

The practice of assuming that the fecal nitrogen excretion is equal to 10 per cent of the nitrogen intake is unsound. Schamberg, Kolmer, Ringer, and Raiziss (43) have pointed out that the nitrogen excretion in the stool is fairly constant regardless of the nitrogen intake. This happens to be about 10 per cent of the normal nitrogen intake, and hence the practice turns out to be reasonably accurate for most experiments. However, for extremes of nitrogen intake considerable error may result from this procedure. For example, the same patient on three consecutive regimes had nitrogen intakes of 2.96, 19.48, and 2.96 gm./24 hours and corresponding fecal nitrogen values of 0.82, 1.12, and 0.75 gm./24 hours, respectively. Another patient (not included in Table 5 and in Figure 2) had nitrogen intakes on consecutive six-day periods of 46.03, 46.03, and 43.92 gm./24 hours and corresponding fecal nitrogen values of 1.42, 2.37, and 1.93 gm./24 hours. In Table 5 and in Figure 2 the analyzed fecal nitrogen excretions of patients on a wide range of nitrogen intakes are seen to be independent of the intake. The data are compiled in part from the authors' analyses and in part from the figures given by Schamberg, *et al.* (43). It will be seen that when extremes of intake are used, a calculated fecal nitrogen figure of 10 per cent introduces a much larger error than the use of the average fecal nitrogen value of 1.283 gm./24 hours. These same conclusions apply to the individual, since a number of the patients in Table 5 and in Figure 2 were studied at several levels of nitrogen intake. Of course, the use of the average fecal nitrogen value may be grossly inaccurate in patients with bowel disturbances associated with diarrhoea.

C. *Derived data*

1. *Theoretical phosphorus, calcium and nitrogen balances*

By a theoretical phosphorus balance is meant that balance which one would expect from the nitrogen and calcium balances. Since about 97 per cent [Shohl (48)] of the

TABLE 5. COMPARISON OF THE ERRORS INTRODUCED BY ASSUMING FECAL NITROGEN TO BE 10% OF THE INTAKE NITROGEN AND BY ASSUMING FECAL NITROGEN TO BE A CONSTANT AVERAGE FIGURE

Source of data	Patient and unit number	Intake of nitrogen gm./24 hr.	Fecal Nitrogen: Analyzed: No. of specimens[18]	Analyzed: gm./24 hr.	Analyzed: % intake (column A)	Calculated as 10% of intake: gm./24 hr.	Calculated as 10% of intake: Deviation from A, %	Calculated as average of analyzed: gm./24 hr.	Calculated as average of analyzed: % intake	Calculated as average of analyzed: Deviation from A, %
(1)	L.C. (438193)	2.956	5	0.792	26.8	0.296	−16.8	1.283	43.4	+16.6
(2)	[19]	2.96	1	1.31	44.2	0.296	−34.2	"	43.2	− 1.0
(2)	[20]	4.15	1	1.28	30.8	0.415	−20.8	"	30.8	0
(2)	Case #8	4.39	1	1.97	44.9	0.439	−34.9	"	29.2	−15.7
(2)	Case #9	4.73	2	1.91	40.4	0.473	−30.4	"	27.1	−13.3
(2)	[21]	5.28	2	1.14	21.6	0.528	−11.6	"	24.3	+ 2.7
(2)	Case #3	6.91	8	1.04	15.1	0.691	− 5.1	"	18.6	+ 3.5
(2)	Case #8	7.06	5	1.19	16.9	0.706	− 6.9	"	18.2	+ 1.3
(2)	Control (Miss W.)	7.24	2	1.35	18.5	0.724	− 8.5	"	17.7	− 0.8
(2)	Case #9	7.44	2	1.49	20.0	0.744	−10.0	"	17.2	− 2.8
(2)	[22]	7.46	1	1.65	22.1	0.746	−12.1	"	17.2	− 4.9
(2)	Case #4	7.53	4	1.04	13.8	0.753	− 3.8	"	17.0	+ 3.2
(2)	Case #5	8.00	4	1.06	13.3	0.800	− 3.3	"	16.0	+ 2.7
(1)	M.A. (168199)	8.10	12	0.843	10.4	0.810	− 0.4	"	15.8	+ 5.4
(1)	F.D. (136431)	9.25	4	1.809	19.6	0.925	− 9.6	"	13.9	− 5.7
(1)	K.P. (369416)	10.313	3	0.852	8.3	1.031	+ 1.7	"	12.4	+ 4.1
(2)	Case #3	11.72	3	1.41	14.8	1.172	− 4.8	"	11.0	− 3.8
(2)	Case #3	11.93	1	0.72	6.1	1.193	+ 3.9	"	10.8	+ 4.7
(2)	Case #9	12.06	2	1.75	14.1	1.206	− 4.1	"	10.6	− 3.5
(2)	Case #5	13.45	1	0.92	6.8	1.345	+ 3.2	"	9.5	+ 2.7
(2)	Case #7	13.71	1	1.22	8.9	1.371	+ 1.1	"	9.4	+ 0.5
(1)	H.B. (425355)	14.99	17	1.341	8.9	1.499	+ 1.1	"	8.6	− 0.3
(1)	K.P. (369416)	15.648	2	1.161	7.4	1.565	+ 2.6	"	8.2	+ 0.8
(1)	I.G. (350260)	16.22	12	1.086	6.7	1.622	+ 3.3	"	7.9	+ 1.2
(1)	L.C. (438193)	18.936	3	0.822	4.3	1.894	+ 5.7	"	6.8	+ 2.5
(1)	L.C. (438193)	19.48	4	1.124	5.8	1.948	+ 4.2	"	6.6	+ 0.8
(2)	Case #3	21.68	6	1.60	7.4	2.169	+ 2.6	"	5.9	− 1.5
(2)	Case #7	24.30	2	1.28	5.3	2.430	+ 4.7	"	5.2	− 0.1
(1)	H.B. (425355)	25.09	10	1.561	6.2	2.509	+ 3.8	"	5.1	− 1.1
(2)	Case #4	27.44	2	0.77	2.8	2.744	+ 7.2	"	4.7	+ 1.9
(2)	Case #5	27.79	1	1.37	4.9	2.779	+ 5.1	"	4.6	− 0.3
(2)	Case #7	28.08	4	2.10	7.5	2.808	+ 2.5	"	4.6	− 2.9
(2)	Case #7	38.65	1	1.36	3.5	3.865	+ 6.5	"	3.3	− 0.2
Average				1.283			± 8.4			± 3.4
Range				0.72 to 2.10			−34.9 to + 7.2			−15.7 to +16.6

(1) Patients studied in our laboratory.
(2) Cases studied or collected from the literature by Schamberg, Kolmer, Ringer, and Raiziss (43).
[18] Specimens are aliquots of 6-day pools for source (1), and usually aliquots of 7-day pools for source (2).
[19] Swen, quoted by Schamberg, et. al. (43).
[20] Case 1 of Hirschfield, quoted by Schamberg, et al. (43).
[21] Klemperer, quoted by Schamberg, et al. (43).
[22] Case 2 of Hirschfield, quoted by Schamberg, et al. (43).

body phosphorus is contained either in bone (calcium to phosphorus ratio = 2.23) or as an integral part of protoplasm (nitrogen to phosphorus ratio of muscle = approximately 14.7), it is possible if one knows the calcium and nitrogen balances to calculate how much of the phosphorus balance can be explained by alterations in these two tissues. For the purpose of these calculations all protoplasm can be considered to have the same composition as muscle, since most of the protoplasm is muscle, and since the N/P ratio in other tis-

sues is not very different from that in muscle [skin, 16.6/1; muscle, 17.2/1; liver,[23] 10.3/1; spleen, 11.1/1; kidney, 12.8/1; and brain,[24] 6.6/1 (1)]. Similarly, it is possible to calculate a theoretical nitrogen balance if one knows the phosphorus and calcium balances, or a theoretical calcium balance if one knows the nitrogen and phosphorus balances.

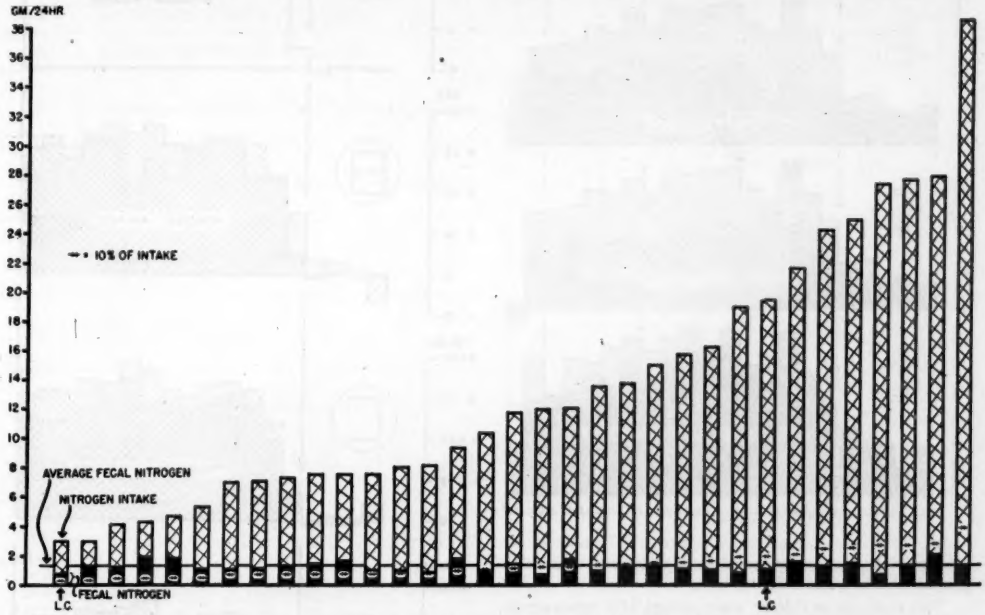

FIG. 2. *Diagram to illustrate that the fecal nitrogen is independent of the level of nitrogen intake*

The scale for intake and fecal excretion of nitrogen in gm./24 hr. is given as the ordinate; the data of a series of individuals are plotted as columns along the abscissa in order of increasing magnitude of intake. The horizontal line extending from zero is the baseline from which all of the divisions of the columns are measured. The total nitrogen intake per day is indicated by the height of the cross-hatched column; the fecal nitrogen per day by the solid column; the average fecal nitrogen value of 1.283 gm./24 hr. by the solid line between the columns; and 10 per cent of the intake by the double pluses in each column. *L. C.*, are the initials of one of a number of individuals studied at more than one level of intake. For the actual measured values see Table 5. The data are compiled in part from the analyses of the authors and in part from the figures given by Schamberg, Kolmer, Ringer and Raiziss (43). The analyzed fecal nitrogen excretions of patients on a wide range of nitrogen intakes are seen to be independent of the intake.

For further discussion, see Text.

In Figure 3 an analysis is made of the phosphorus, calcium and nitrogen balances during a metabolic experiment in which marked fluctuations of the balances were induced by starting and stopping testosterone propionate therapy. This figure illustrates that the phosphorus balances very nearly can be accounted for by the calcium and nitrogen balances during both the control observations and the experimental periods. During the 22 five-day periods 29.1 gm. of phosphorus were retained; 14.2 gm. could be accounted for by the calcium retention, and 18.1 gm. by the nitrogen retention; thus, of the 29.1 gm. of phosphorus retained 32.3 gm. were explained by both the calcium and the nitrogen retentions.[25] The small discrepancy between the

[23] The ratio of liver is affected by phosphorus present in glycogen.

[24] The ratio of brain is affected by phosphorus present in phospholipids.

[25] These figures were obtained from the following calculations:

CALCULATIONS	*22 five-day periods*
Phosphorus retained	29.1 grams
Calcium retained	31.7 grams
Theoretical phosphorus based on calcium [calcium ×1/2.23 (48)]	14.2 grams
Nitrogen retained	266.4 grams
Theoretical phosphorus based on nitrogen [nitrogen×1/14.7 (13)]	18.1 grams
Theoretical phosphorus based on calcium and nitrogen	32.3 grams

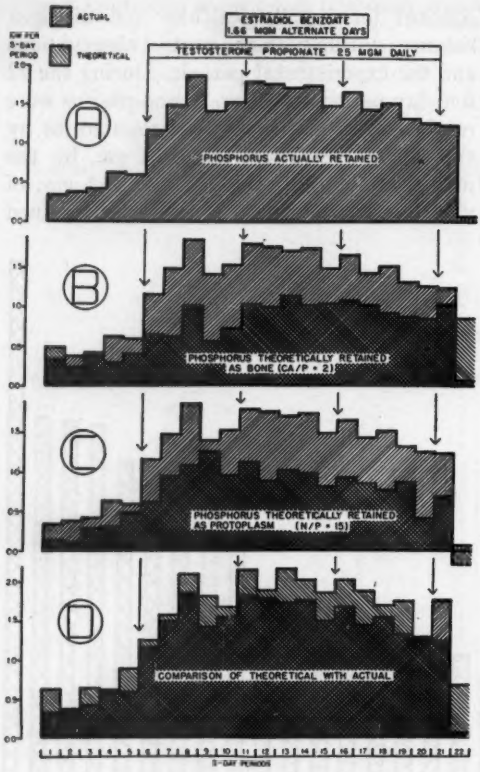
ACTUAL
THEORETICAL
ESTRADIOL BENZOATE 1.66 MGM ALTERNATE DAYS
TESTOSTERONE PROPIONATE 25 MGM DAILY
PHOSPHORUS ACTUALLY RETAINED
PHOSPHORUS THEORETICALLY RETAINED AS BONE (CA/P = 2)
PHOSPHORUS THEORETICALLY RETAINED AS PROTOPLASM (N/P = 15)
COMPARISON OF THEORETICAL WITH ACTUAL
5-DAY PERIODS

FIG. 3. *An analysis of the calcium, phosphorus and nitrogen balances in a metabolic experiment.*

This study on an elderly, male patient with osteoporosis (*M. H., M. G. H. #267511*) consists of 22 five-day periods. The ordinate is the scale for balance in grams per five-day period; the abscissa is the scale for five-day periods. The horizontal line starting at zero of the ordinate is the baseline; balances extending from the baseline toward the top of the chart are positive; those extending from the baseline toward the bottom of the chart are negative.

The chart has four divisions: A—the measured phosphorus balance; B—the measured phosphorus balance with superimposed theoretical balance explainable by the measured calcium balance (Ca/P=2); C—the measured phosphorus balance with superimposed theoretical phosphorus balance explainable by the measured nitrogen balance (N/P=15); and D—the measured phosphorus balance with superimposed theoretical phosphorus balance explainable by both the measured calcium and the measured nitrogen balances, i.e. a summation of B and C. It will be noted in D that the actual phosphorus retention very closely follows the theoretical phosphorus retention. As a matter of fact, if a ratio of Ca/P of 2.23 instead of 2 had been used in constructing the chart, the discrepancy would be very much less. This experiment supports the contention that nearly all of the phosphorus retained as a result of testosterone propionate therapy is retained either as bone or as protoplasm.

For further discussion, see text. Additional data on this patient are published elsewhere (1, 39).

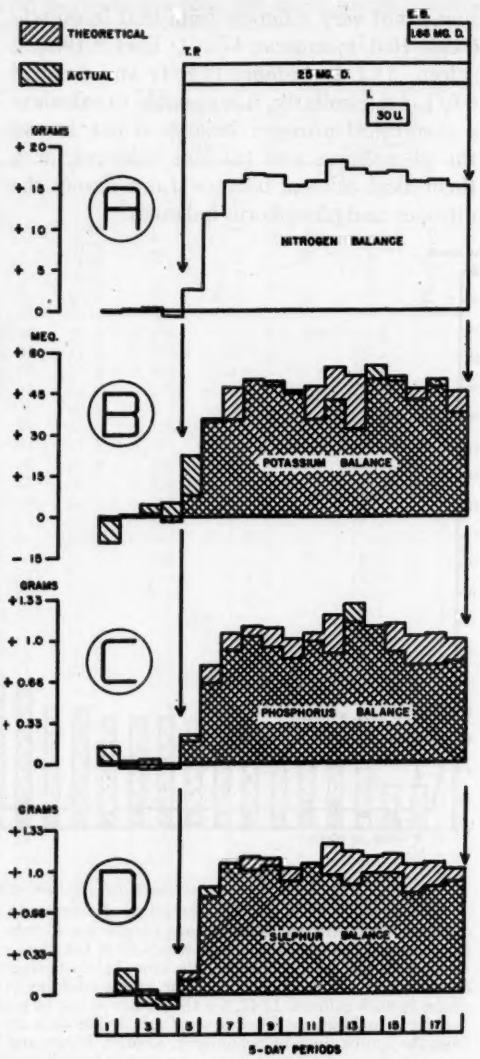

FIG. 4. *A comparison of the deviations in the nitrogen, potassium, phosphorus, and sulfur balances as a result of therapy*

The subject of this analysis was a female patient with Cushing's Syndrome (*B. V., M. G. H. #74372*). The scales for balances in amounts per five-day period are given as the ordinates; the scale of five-day periods is given as the abscissa. The analysis consists of 18 five-day periods. The horizontal line starting at zero on each ordinate is the baseline of that particular balance; balances extending from the baselines toward the top of the chart are positive; those extending from the baselines toward the bottom of the chart are negative. The balances are charted as deviations from the average of the control periods rather than as the

TABLE 6. FACTORS USED IN DERIVING CERTAIN COMPONENTS OF MUSCLE PROTOPLASM FROM NITROGEN (N)

Factor Number	Component to be Derived	Final Unit	Factor[26]	Source of Factor
1.	Protein in Protoplasm	gm.	6.25	—
2.	Protoplasm: fat-free but not extracellular-fluid free	gm.	32	33.4;[27] 31.6.[28]
3.	Protoplasm: fat-free and extracellular-fluid-free ("true muscle" (50))	gm.	27	27.7;[27] 26.5.[28]
4.	Intracellular Fluid in Protoplasm	cc.	19	factor 2×0.60 [0.595;[29] 0.63[28]].
5.	Extracellular Fluid in Protoplasm	cc.	5	factor 2×0.16 [0.17;[29] 0.16[28]].
6.	Potassium in Intracellular Fluid in Protoplasm	m. eq.	2.7	factor $4\times\frac{143^{28}}{1000}$.
7.	Sodium in Extracellular Fluid of Protoplasm	m. eq.	0.77	factor $5\times\frac{151^{30}}{1000}$.
8.	Phosphorus in Protoplasm	gm.	$\frac{1}{15}$	14.7.[31]
9.	Sulfur in Protoplasm	gm.	$\frac{1}{14.5}$	15.7;[31] 14.3.[32]
10.	Fat Calorically-Equivalent to Protoplasm	gm.	2.8	factor $1\times4.1\times\frac{1}{9.3}$.
11.	Protoplasm (fat-free) Minus Fat Calorically-Equivalent to Protoplasm	gm.	29.2	factor 2—factor 10.

[26] Factor×nitrogen in grams=component.
[27] Factor calculated from Hastings and Eichelberger (33), and based on 314 grams of protein per liter of intracellular water (32).
[28] Factor from Harrison, Darrow and Yannet (32).
[29] Factor from Hastings and Eichelberger (33).
[30] 151 is based on the assumption (53) that the serum protein is 7.5 gm. per 100 cc. and the serum sodium, 140 m. eq. /liter. To convert sodium in terms of serum water, one employs the formula of Van Slyke, Wu and MacLean (53): serum water per liter of serum=990—0.8 P, where P is the grams of protein per liter of serum.
[31] Factor from Benedict (13).
[32] Factor from Wilson (56).

measured and the theoretical phosphorus balances would be eliminated by a 10 per cent error in the intake of calcium, phosphorus or nitrogen. This analysis supports the contention that nearly all of the phosphorus retained as a result of testosterone propionate therapy is retained either as bone or as protoplasm. Further illustrations of analyses of the calcium, phosphorus and nitrogen balances will be given below (see Figures 4 and 5).

Calculations such as the above have two purposes: 1) to detect any gross errors in the

deviations actually measured. T.P.=testosterone propionate; I=insulin; E. B.=estradiol benzoate; D.=dosage per day; U.=units per day. The data for potassium are based on analyses of urinary excretions alone; the fecal potassium excretion was assumed to be 8 per cent of the potassium intake. The factors for the calculations are given in Table 6.

The chart has four divisions: A—the measured nitrogen balance; B—the measured nitrogen balance with superimposed theoretical nitrogen balance explainable by the measured potassium balance; C—the measured nitrogen balance with superimposed theoretical nitrogen balance explainable by the measured phosphorus balance (after the phosphorus theoretically retained with calcium had been subtracted as in Figure 3); and D—the measured nitrogen balance with superimposed theoretical nitrogen balance explainable by the measured sulfur balance. It will be seen that there is a close correspondence between the measured and the theoretical nitrogen balances. This is evidence that testosterone propionate therapy induced a retention of nitrogen, potassium, phosphorus, and sulfur in the proportions that exist in muscle protoplasm.

For further discussion, see text. Additional data on this patient are published elsewhere [(1), and *case 1* in (7)].

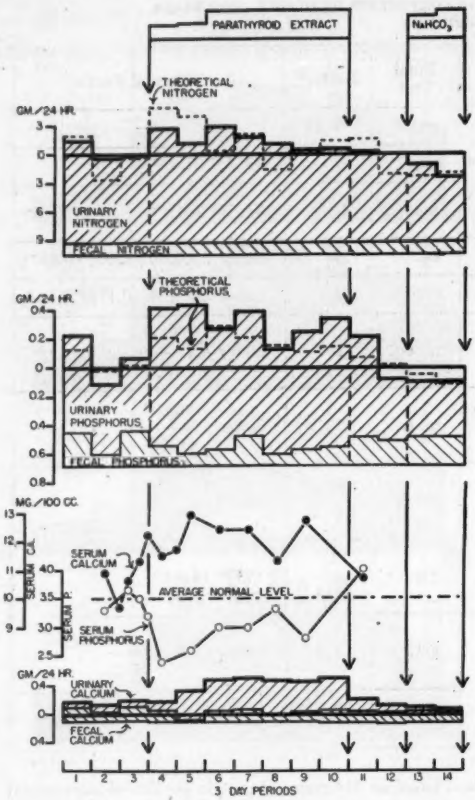

FIG. 5. *A comparison of the deviations of the calcium, phosphorus and nitrogen balances as a result of therapy*

The study was done on a 45 year old man with myositis ossificans (*W. S., M. G. H. #281254*). He was studied for 14 three-day periods. The nitrogen, phosphorus and calcium balances are constructed as described in Figure 1. The theoretical nitrogen balance is constructed from the measured phosphorus balance (after the phosphorus theoretically retained with calcium has been subtracted as described in Figure 3). The theoretical phosphorus balance is constructed from the measured nitrogen and calcium balances as in Figure 3. Injections of parathyroid extract (250 to 500 units per day) were given from the 4th to the 11th period inclusive.

It will be seen that the theoretical phosphorus balance does not agree well with the determined phosphorus balance; too much phosphorus was excreted, particularly in periods 4 and 5. Furthermore, this excretion occurred at a time when there was very little change in the calcium balance. From observations and calculations such as these it was shown by Albright, Bauer, Ropes, and Aub (3) that one of the first actions of the parathyroid hormone is to cause excretion of phosphorus in the urine. Additional data on this patient are to be found elsewhere [*Case 2* in (2) and *Case 8* in (3)].

For further discussion, see text.

data (*vide supra*), and 2) to emphasize any situation where the measured balances differ from the theoretical balances. To illustrate this second point, it was through calculations such as these that Albright, Bauer, Ropes and Aub (3) showed that one of the first actions of the parathyroid hormone is to cause a urinary excretion of phosphorus from a source other than bone or protoplasm (see Figure 5); this source most probably was the inorganic phosphorus of body fluids (see Figure 6).

2. *Theoretical potassium and sulfur balances*

If the data include measurements of the balances of potassium and sulfur (both constituents of protoplasm), it is possible likewise to derive theoretical nitrogen balances from the balances of each of these minerals to compare with the determined nitrogen bal-

FIG. 6. *Thirty-one day fast of patient studied by Benedict* (13)

A. *An analysis of nitrogen, phosphorus, potassium and sulfur excretions*

The scale of excretion in gm./24 hr. is given as the ordinate; the scale of days and periods is given as the abscissa. Excretions for each substance are charted upward from the baseline and are superimposed on each other. Since the patient had virtually no fecal excretion, the chart is constructed entirely from the urinary values. The data for the first day are charted separately; thereafter, all data are charted in three-day periods. All values shown are amounts per 24 hours. The measured nitrogen balance (black block) is compared with the "theoretical-nitrogen-balance-based-on-phosphorus (not explainable by the calcium balance)" (dash line), the "theoretical-nitrogen-balance-based-on-potassium" (dot line), and the "theoretical-nitrogen-balance-based-on-sulfur" (solid line). For factors, see Table 6.

It will be seen that all of the theoretical balances agree well with the measured nitrogen balance except during the first seven days, when too much phosphorus and potassium were lost. The discrepancy during these seven days is probably due to loss of glycogen. This analysis indicates that the factors of Table 6 that are employed in determining the ratios of nitrogen to phosphorus, to potassium, and to sulfur are reasonably accurate.

For further discussion, see text.

B. *Comparison between measured weight loss and theoretical weight loss*

The scale of weight loss in Kg. is given as the ordinate; the scale of days and periods is given as the abscissa. The actual weight curve is shown by the solid line with large black dots. The "theoretical weight curve" based on nitrogen alone is shown by the solid line with crosses; that based on nitrogen corrected for sodium is shown by the solid line with the black triangles; and that based on nitrogen corrected for both sodium and potassium is shown by the solid line with black squares. The "caloric discrepancy" curve based on the nitrogen corrected for both the sodium and the potassium is shown by the broken line with the black squares.

For discussion, see text.

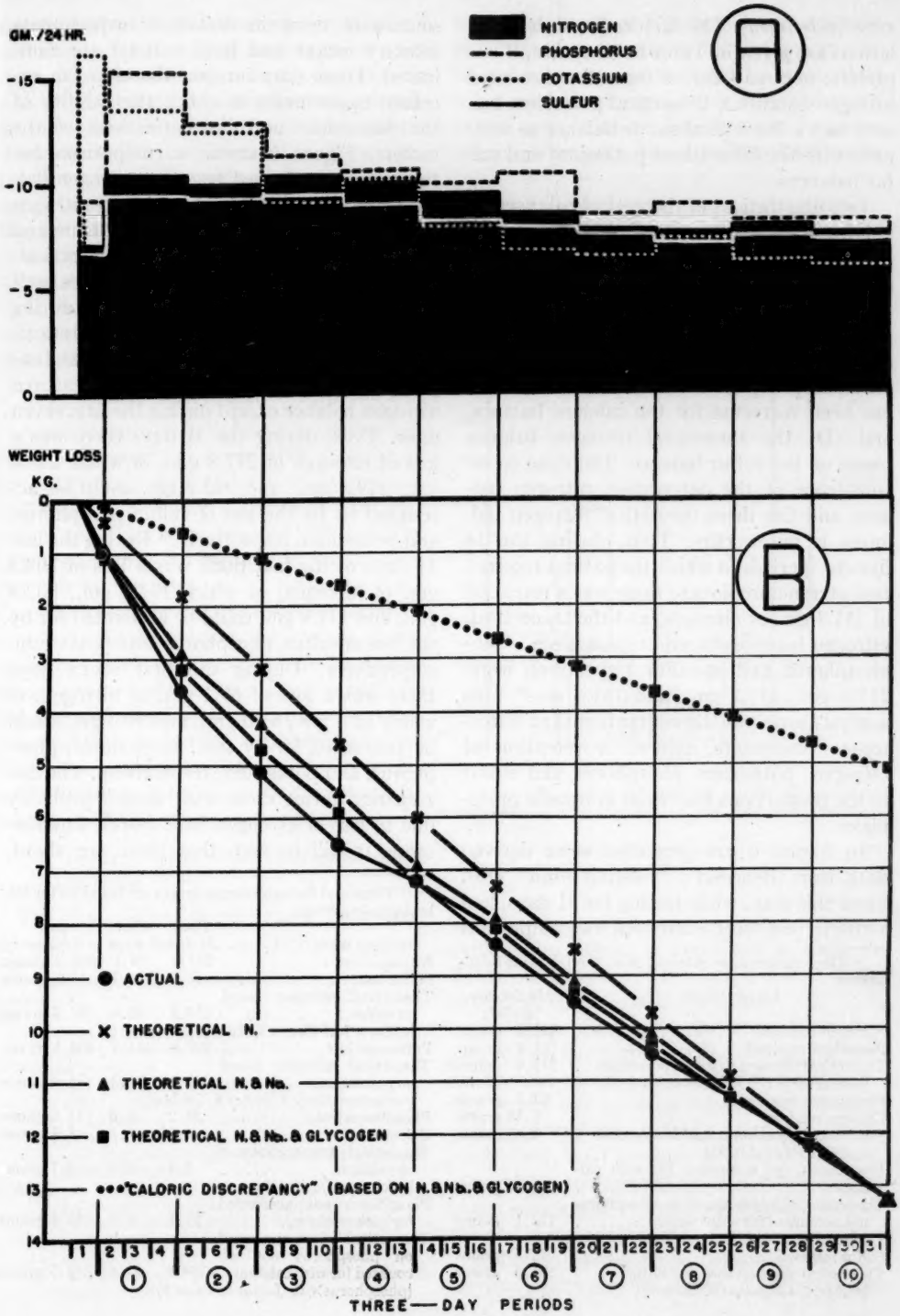

FIG. 6. *(See opposite page for description.)*

ance (*vide infra*). The factors for such calculations are given in Table 6. Of course, if one prefers, one may derive from the measured nitrogen balance a theoretical potassium balance and a theoretical sulfur balance to compare with the determined potassium and sulfur balances.

Two illustrations of theoretical balances involving potassium and sulfur are presented. In Figure 4 are charted (A) the nitrogen balance resulting from a therapeutic agent (testosterone propionate), (B) the theoretical nitrogen balance based on the potassium balance, (C) the theoretical nitrogen balance based on phosphorus balance after the latter has been corrected for the calcium balance, and (D) the theoretical nitrogen balance based on the sulfur balance. The close correspondence of the determined nitrogen balance and the three theoretical nitrogen balances is noteworthy. Thus, during the 14 five-day periods in which the patient received testosterone propionate there was a retention of 211.3 gm. of nitrogen, and the theoretical-nitrogen-balances-based-on-potassium, on-phosphorus, and on-sulfur, respectively were: 217.6 gm., 173.1 gm., and 181.3 gm.[33] This analysis supports the contention that testosterone propionate induced a retention of nitrogen, potassium, phosphorus and sulfur in the proportions that exist in muscle protoplasm.

In Figure 6 are presented some derived data from Benedict's "Fasting Man" (13). Since this man, while fasting for 31 days, had virtually no fecal excretion, two important sources of error in metabolic experiments (dietary intake and fecal output) are eliminated. These data furnish, therefore, an excellent opportunity to check the validity of the deductions and the correctness of the factors. Figure 6 shows a comparison between the determined negative nitrogen balance and the theoretical negative nitrogen balances based on phosphorus, potassium and sulfur. It will be noted that the theoretical-nitrogen-balance-based-on-sulfur agrees well with the measured nitrogen balance during the entire experiment; that the theoretical-nitrogen-balances-based-on-potassium and on-phosphorus agree well with the measured nitrogen balance except during the first seven days. Thus, during the 31 days there was a loss of nitrogen of 277.3 gm., of which 253.8 gm., 319.5 gm., and 262.2 gm. could be accounted for by the loss of sulfur, phosphorus, and potassium, respectively.[34] During the last 24 days of the fast there was a loss of 208.2 gm. of nitrogen, of which 191.4 gm., 232.5 gm., and 171.9 gm. could be accounted for by the loss of sulfur, phosphorus, and potassium, respectively. During the first seven days there was a loss of 69.1 gm. of nitrogen, of which 62.4 gm., 87.0 gm., and 90.3 gm. could be accounted for by the loss of sulfur, phosphorus, and potassium, respectively. The discrepancy during these seven days is probably due to loss of glycogen (*vide infra*). Furthermore, it will be seen that there are slight,

[33] These figures were obtained from the following calculations:

CALCULATIONS	*14 five-day periods*	
Nitrogen retained	211.3	grams
Potassium retained	587.5	m. eq.
Theoretical nitrogen based on potassium [potassium×1/2.7 (factor 6, Table 6)]	217.6	grams
Phosphorus retained	12.2	grams
Calcium retained	1.48	grams
Theoretical phosphorus based on calcium [calcium×1/2.23(38)]	0.66	grams
Phosphorus not accounted for with calcium	11.54	grams
Theoretical nitrogen based on phosphorus not accounted for with calcium [phosphorus×15 (factor 8, Table 6)]	173.1	grams
Sulfur retained	12.5	grams
Theoretical nitrogen based on sulfur [sulfur×14.5 (factor 9, Table 6)]	181.3	grams

[34] These and the subsequent figures are based on the following calculations:

CALCULATIONS	*Total 31 days*	*First 7 days*	*Last 24 days*
Nitrogen lost	277.3	69.1	208.2 grams
Sulfur lost	17.5	4.3	13.2 grams
Theoretical nitrogen based on sulfur [sulfur×14.5 (factor 9, Table 6)]	253.8	62.4	191.4 grams
Potassium lost	707.8	243.7	464.1 m. eq.
Theoretical nitrogen based on potassium [potassium×1/2.7 (factor 6, Table 6)]	262.2	90.3	171.9 grams
Phosphorus lost	24.2	6.6	17.6 grams
Calcium lost	6.5	1.7	4.8 grams
Theoretical phosphorus based on calcium [calcium×1/2.23 (48)]	2.9	0.8	2.1 grams
Phosphorus not accounted for with calcium	21.3	5.8	15.5 grams
Theoretical nitrogen based on phosphorus not accounted for with calcium [phosphorus×15 (factor 8, Table 6)]	319.5	87.0	232.5 grams

constant errors in the sulfur and the phosphorus balances which do not modify the conclusions. These errors may be in the factors with which the theoretical balances are calculated, or more probably in the measured values themselves. The usefulness of such calculations in indicating constant errors is apparent. This analysis indicates, therefore, that the deductions concerning the relationships of the nitrogen, phosphorus, calcium, potassium and sulfur to each other in muscle protoplasm and in bone are reasonably valid, and that the factors employed in calculating these relationships are reasonably accurate.

3. *Theoretical weight curve*

Attention is first called to the marked differences in weight between 1000 Calories of fat (108[35] gm.), 1000 Calories of protoplasm (1248[35] gm.), and 1000 Calories of "wet glycogen" (581[35] gm.) (see Figure 7). It should be emphasized that the burning of fat involves the loss to the body of fat only; the fatty tissue in which the fat was deposited remains intact. On the other hand, the burning of glycogen or protein involves not only the loss of the glycogen or of the protein, but in addition the loss of considerable quantities of intra- and extracellular water. It follows that an individual could lose 10,000 Calories of fat and still gain weight, if, at the same time, he gained 1,000 Calories of protoplasm.

a. *Concepts upon which theoretical weight curve is based*

The "theoretical weight curve" has been devised as a means of analyzing in a prolonged metabolic study on a constant intake to what extent various fluctuations in the weight curve of a patient are to be ascribed to accountable changes, especially to changes in nitrogen balance. Furthermore, any discrepancy between the actual weight curve and the theoretical weight curves invites an explanation. Does it represent a shift in fluids? In glycogen? Or what? Or is it to be explained by an error in the data?

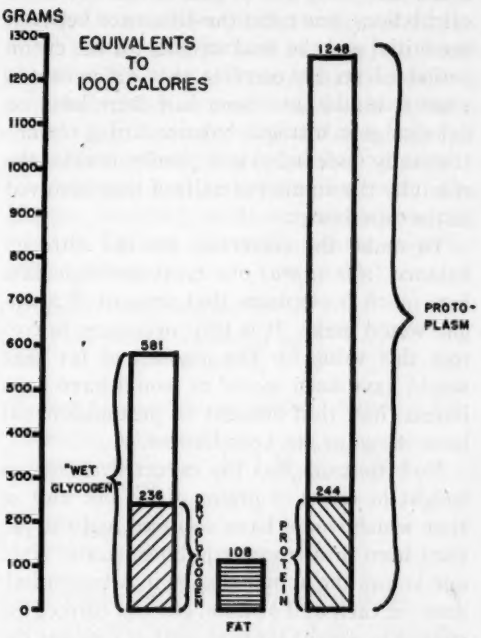

FIG. 7. *Diagram to illustrate the marked differences between the weights of glycogen, fat and protoplasm equivalent to 1000 calories*

The calculations for the figures in this diagram are given in footnote thirty-five([35]).

To obtain this curve one must know the weights at the beginning and the end of the study, and the nitrogen balances for the entire study and for each subdivision (six-day period, three-day period, one-day period, etc.). One must assume a constant caloric output and the same state of hydration, glycogen saturation, etc. at the beginning and at the end of the analysis.

It is first necessary to determine the discrepancy between the caloric intake and the

[35] These figures are obtained from the following calculations:

Fat

9.3 Calories = 1 gram of fat.
∴ 1,000 Calories = 108 grams of fat.

Protein and protoplasm

4.1 Calories = 1 gram of protein.
∴ 1,000 Calories = 244 grams of protein.
244 grams of protein = 39 grams of nitrogen (factor 1, Table 6).
∴ 39 grams of nitrogen = 1248 grams of protoplasm (factor 2, Table 6).

Glycogen

4.23 Calories = 1 gram of "dry glycogen" (48).
∴ 1,000 Calories = 236 grams of "dry glycogen."
One gram of "dry glycogen" = 2.46 grams of "wet glycogen" (21).
∴ 236 grams of "dry glycogen" = 581 grams of "wet glycogen."

caloric output and to express this "caloric discrepancy" in grams of fat per unit of time; to determine, in other words, what the weight loss would have been had the individual remained in nitrogen equilibrium so that the caloric discrepancy would have been entirely taken care of by loss or gain of fat. For these calculations, one takes the difference between the initial and the final weights for the entire period of study, corrects this difference to what it would have been had there been no net change in nitrogen balance during the entire study (*vide infra*) and, finally, divides the result by the number of units of time involved in the experiment.

To make the correction for the nitrogen balance (*vide supra*) one must first calculate how much protoplasm that amount of nitrogen would make. It is then necessary to correct this value for the amount of fat that would have been stored or would have been burned had that amount of protoplasm not been stored or not been burned.

Now that one has the expected change in weight in terms of grams of fat per unit of time which would have occurred had the patient been in nitrogen equilibrium, and since one knows the actual change in nitrogen balance for each unit of time, one can correct the change in weight for each unit of time for the nitrogen balance by using the same calculations which were used in the preceding paragraph. In such a manner, one obtains the theoretical weight change for each unit of time, and from these one calculates the theoretical weight curve.

It should be noted that any constant error in the metabolic data (*e.g.*, an error in the nitrogen intake) will affect the "caloric discrepancy" calculation but not the theoretical weight curve; this does not apply, of course, to an error in a factor used in making those calculations which vary from period to period (*e.g.*, the factor for calculating protein from nitrogen).

It should be further noted that the above calculations become more accurate the longer the duration of the study, since any discrepancy introduced by a difference in the amount of hydration between the beginning and the end of the experiment is minimized. By the same token, it is desirable to choose as points for the beginning and the ending of such an analysis times when the water balance and the glycogen content of the body are in equilibrium or in the same state of non-equilibrium.

If one's metabolic data include sodium or chloride balances in addition to the nitrogen balances one can correct the theoretical weight curve for alterations in extracellular fluid volume. In these calculations (*vide infra*) it is assumed that there is no sodium or chloride in intracellular fluid [the chloride of erythrocytes is insignificant (37)]; furthermore, the sodium derived from the skeleton can be neglected. One follows the same steps; one first determines the sodium or chloride for the entire experiment; one then calculates the amount of extracellular fluid involved; one then corrects this extracellular fluid volume for that moiety of extracellular fluid already taken into account in the calculations for the nitrogen balance; one then corrects the final weight at the end of the experiments (already corrected for what it would have been had the subject been in nitrogen equilibrium) for what it would have been had there been no change in extracellular fluid volume; one then calculates a new caloric discrepancy from this new doubly corrected final weight and the initial weight; finally from the sodium or chloride balance of each subdivision of the experiment, one calculates in the same way the extracellular fluid volume not accounted for by the nitrogen calculations and adds this correction to the nitrogen correction in determining the theoretical weight change for each period. It should be emphasized that an error, such as, loss of NaCl in sweat, if constant, will affect the caloric discrepancy but not the theoretical weight curve.

Since the chloride method of measuring the extracellular fluid volume gives a smaller volume than either the sodium or sulfocyanate methods [Winkler, Elkinton, and Eisenman (57)], its use is probably preferable to the use of sodium for these calculations providing the chloride concentration of the

serum remains fairly constant. One must use the same serum chloride concentration to calculate the total change in extracellular fluid volume as one uses to calculate the change for each subdivision. In this respect the problem differs from that of determining the change in extracellular fluid volume at any one period of time [Lavietes, D'Esopo, and Harrison (37)].

If one's metabolic data include potassium balances in addition to the nitrogen and sodium (or chloride) balances, one can correct the theoretical weight curve for alterations in intracellular fluid volume not already accounted for by changes in the nitrogen balance; *i.e.*, for intracellular hydration or dehydration [Elkinton and Winkler (18)]. The steps are the same. The small amount of potassium in extracellular fluid is neglected.

b. *Calculations*

The calculations and the data involved in the construction of a "theoretical weight curve" are divided into two groups: a) those that pertain to the entire study as a whole, and b) those that pertain to each individual period of the study. Preliminary calculations need to be made for each of these two groups; however, Preliminary Calculations C and D are carried out only if the data include sodium (or chloride) and potassium balances. The factors employed in these calculations are given in Table 6. An outline of the steps involved follows:

Preliminary Calculation A: *Changes in Body Weight*

$$\left[\begin{matrix}\text{Body weight in grams at beginning}\\ \text{of study (or period)}\end{matrix}\right] - \left[\begin{matrix}\text{Body weight in grams at end}\\ \text{of study (or period)}\end{matrix}\right]$$

Preliminary Calculation B: *Changes in Weight of Protoplasm Corrected for an Isocaloric Amount of Fat*

$$\left[\left(\begin{matrix}\text{Nitrogen balance in}\\ \text{grams for study}\\ \text{(or period)}\end{matrix}\right)\times 32\left(\begin{matrix}\text{factor 2,}\\ \text{Table 6}\end{matrix}\right)\right] - \left[\left(\begin{matrix}\text{Nitrogen balance in}\\ \text{grams for study}\\ \text{(or period)}\end{matrix}\right)\times 2.8\left(\begin{matrix}\text{factor 10,}\\ \text{Table 6}\end{matrix}\right)\right]$$

or, simplified:

$$\left[\begin{matrix}\text{Nitrogen balance in grams}\\ \text{for study (or period)}\end{matrix}\right]\times\left[29.2\left(\begin{matrix}\text{factor 11,}\\ \text{Table 6}\end{matrix}\right)\right]$$

To be carried out only if the data include sodium (or chloride) and potassium balances:

Preliminary Calculation C: *Changes in Weight of Extracellular Fluid Not Accounted For With Protoplasm*

$$\left[\left(\begin{matrix}\text{Sodium balance in m.eq.}\\ \text{for study (or period)}\end{matrix}\right)\div 151^{36}\times 1000\right] - \left[\left(\begin{matrix}\text{Nitrogen balance in grams}\\ \text{for study (or period)}\end{matrix}\right)\times 5\left(\begin{matrix}\text{factor 5,}\\ \text{Table 6}\end{matrix}\right)\right]$$

or:

$$\left[\left(\begin{matrix}\text{Chloride balance in m.eq.}\\ \text{for study (or period)}\end{matrix}\right)\div 104^{37}\times 1000\right] - \left[\left(\begin{matrix}\text{Nitrogen balance in grams}\\ \text{for study (or period)}\end{matrix}\right)\times 5\left(\begin{matrix}\text{factor 5,}\\ \text{Table 6}\end{matrix}\right)\right]$$

Preliminary Calculation D: *Changes in Weight of Intracellular Fluid and/or Glycogen Not Accounted for With Protoplasm*

$$\left[\left(\begin{matrix}\text{Potassium balance in m.eq.}\\ \text{for study (or period)}\end{matrix}\right)\div 143^{38}\times 1000\right] - \left[\left(\begin{matrix}\text{Nitrogen balance in grams}\\ \text{for study (or period)}\end{matrix}\right)\times 19\left(\begin{matrix}\text{factor 4,}\\ \text{Table 6}\end{matrix}\right)\right]$$

Final Calculation A: *"Caloric Discrepancy"*

$$\left[\begin{matrix}\text{Preliminary Calculation A}\\ \text{for entire study}\end{matrix}\right] - \left[\begin{matrix}\text{Sum of Preliminary Calculations}\\ \text{B, C, and D for entire study}\end{matrix}\right] \div \left[\text{Number of Periods}\right]$$

Final Calculation B: *Theoretical Weight Change*

$$\left[\text{Final Calculation A}\right] + \left[\begin{matrix}\text{Sum of Preliminary Calculations B, C, and D for the period}\end{matrix}\right]$$

[36] 151 m. eq. of sodium to 1 liter of extracellular fluid (53); see footnote to Table 6.

[37] 104 m. eq. of chloride to 1 liter of extracellular fluid (49); or one may calculate for each patient using the formula: Cl in serum in m. eq. $\times \frac{0.95}{0.93}$ (18).

[38] 143 m. eq. of potassium to 1 liter of intracellular fluid (32).

The calculations in regard to potassium are subject to error if there is a shift in the body glycogen. Fenn (20, 21) has shown that there is 1 m. eq. of potassium in 6.7, 8.5, and 6.8 grams of "wet glycogen"[39] in the rat, cat, and dog, respectively. Thus, a potassium loss not accounted for by loss of protoplasm as calculated from the nitrogen balance may represent either a loss of glycogen (1 m. eq. of potassium = 6.8 grams, if one takes the figure for the dog) or dehydration with respect to intracellular fluid (1 m. eq. of potassium = 7.04[40] grams). The authors know of no way of differentiating between intracellular dehydration and loss of glycogen except by the nature of the experiment. Fortunately, since the factors are so nearly the same, the weight loss will be nearly the same whether the extra potassium is considered to have been derived from intracellular water or from glycogen.

c. *Illustrations*

Three illustrations of theoretical weight charts are presented. The first (Figure 6B) concerns Benedict's "Fasting Man" (13). Since, as mentioned above, the data in this experiment are subject to fewer errors, they are useful in ascertaining the validity of the factors. At the same time this experiment is not ideal for an analysis of the theoretical weight curve, because there was no subsequent recovery or control period during which the subject regained his initial equilibria of fluid and glycogen. This defect is offset in this particular instance by the fact that data of the sodium, potassium and glycogen balances are available to permit correction of the "caloric discrepancy" for these factors.

It will be noted in Figure 6B that the final "theoretical weight curve" (based on all of the corrections) and the measured weight curve coincide very nicely. Since from the nature of the experiments, glycogen was undoubtedly lost during the first seven days, the potassium loss not accounted for by the nitrogen loss during this period of time probably is accounted for by loss of glycogen rather than additional intracellular fluid (intracellular dehydration). If Benedict's figures for "dry glycogen" as obtained by indirect calorimetry are converted into "wet glycogen," there was a loss of 415 grams during the first seven days; this result compares favorably with that of 390 grams obtained by the authors by calculation from the potassium loss.[41]

This chart illustrates the success with which the changes in weight as determined by weighing the patient can be accounted for by changes in nitrogen, sodium and potassium. It indicates that the factors in Table 6 (which are employed in these calculations) are reasonably accurate.

The second illustration of the theoretical weight chart (Figure 8) is based on a study of the effect of testosterone compounds on a man with hyperthyroidism (36). Since the theoretical weight curve in this case was not corrected for sodium and potassium balances, the difference between it and the measured weight curve is presumably due to fluctuations in fluid not accounted for with protoplasm. It will be seen that during the administration of the testosterone compounds there

[39] One gram of "dry glycogen" = 2.46 grams of "wet glycogen" (21).

[40] This figure is obtained from the following calculations:
Nitrogen = 1/2.7 m. eq. of potassium in intracellular fluid of protoplasm (factor 6, Table 6).
Nitrogen = 1/19 cc. of intracellular fluid in protoplasm (factor 4, Table 6).
∴ 1/2.7 m. eq. of potassium = 1/19 cc. of intracellular fluid
or
One m. eq. of potassium = 2.7/19 = 7.04 cc. of intracellular fluid.
One cc. of intracellular fluid is assumed to weigh 1 gram.
∴ One m. eq. of potassium = 7.04 grams of intracellular fluid.

[41] These figures are obtained from the following calculations:
Glycogen
During the first seven days 168.7 grams of "dry glycogen" were lost (13).
One gram of "dry glycogen" = 2.46 grams of "wet glycogen" (21).
168.7 × 2.46 = 415 grams of "wet glycogen."
Potassium
During the first seven days 69.1 grams of nitrogen were lost (13).
69.1 grams of nitrogen = 186.6 m. eq. of potassium (factor 6, Table 6).
During the first seven days 243.0 m. eq. of potassium were lost (13).
∴ 243.0 − 186.6 = 57.4 m. eq. of potassium not accounted for by nitrogen balance.
One m. eq. of potassium = 6.8 grams of "wet glycogen" (21).
∴ 57.4 × 6.8 = 390 grams of "wet glycogen."

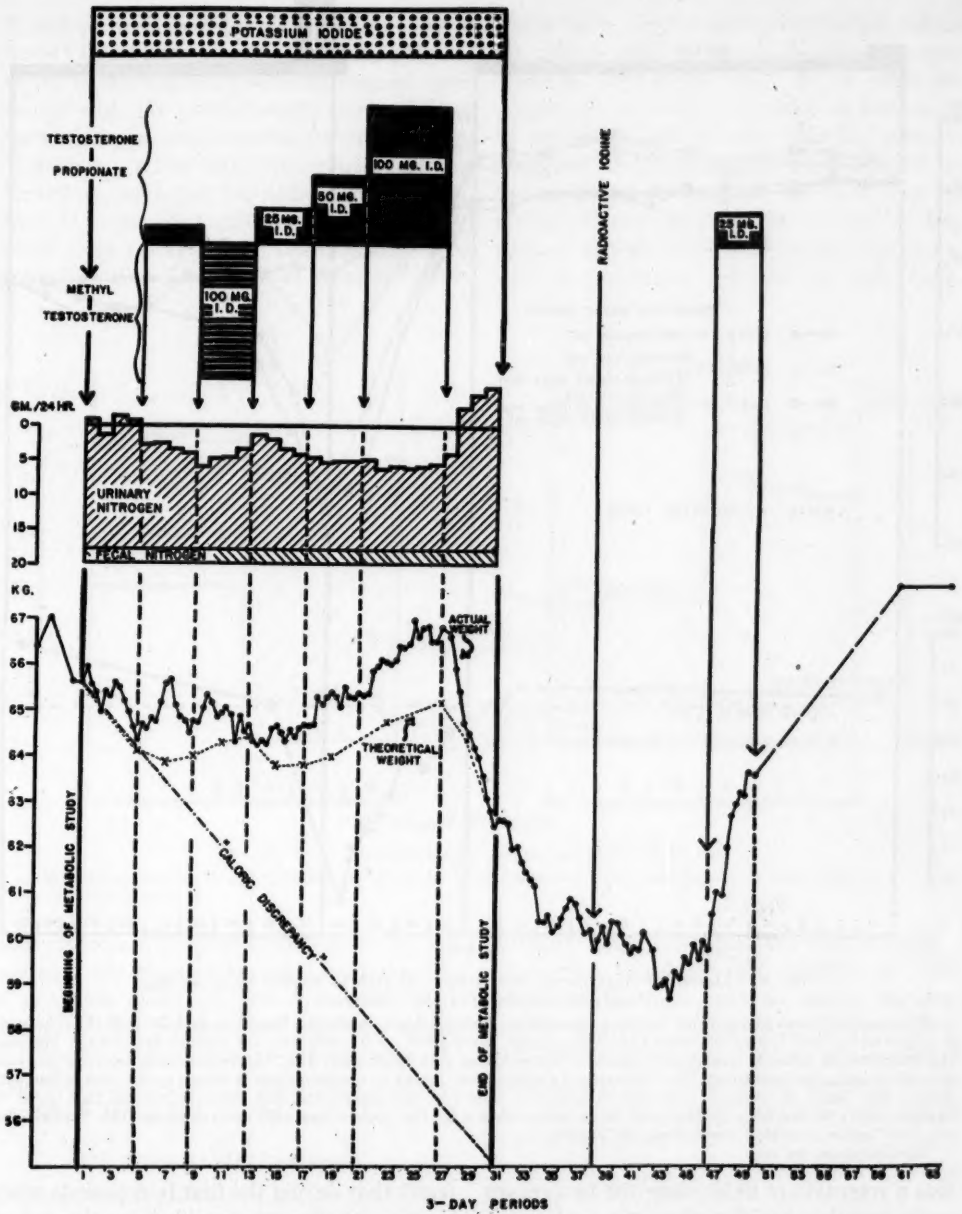

FIG. 8. *Diagram to illustrate the "theoretical weight curve" on a patient with hyperthyroidism under testosterone propionate and methyl testosterone therapy*

This analysis is on Patient *P. C., M. G. H. #369235*. The scales for nitrogen intake and balance in gm./24 hr. and for weight in Kg. are given as the ordinates; the scales for three-day periods as the abscissa. The analysis covers 30 three-day-periods. The diagram of the nitrogen data is constructed as indicated in Figure 1. The fecal nitrogen was assumed to be 10 per cent of the nitrogen intake (see Figure 2). The "theoretical weight curve" in this case is not corrected for sodium and potassium balances. Additional data on this patient are published elsewhere [*Case 3* in (36)].

For discussion, see text.

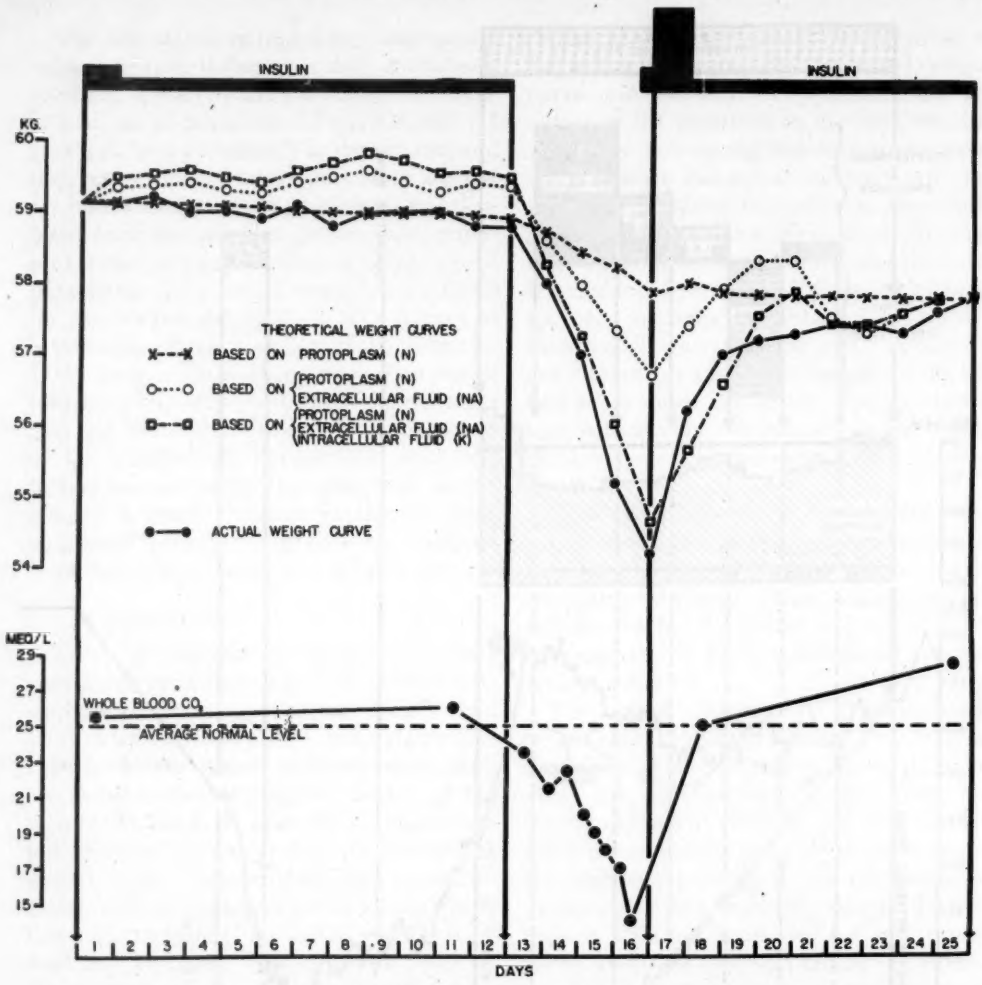

FIG. 9. *"Theoretical weight curve" on a patient with diabetes mellitus before, during, and after a period of insulin withdrawal*

This analysis is calculated from the data reported by Atchley, Loeb, Richards, Benedict, and Driscoll (8). The scale of weight in Kg. and the whole blood CO_2 in m. eq. per l. are given on the ordinate; the scale in days on the abscissa. The weight curve actually measured is shown as the solid line with black dots. The "theoretical weight curves" are constructed as indicated in the text. The "theoretical weight curve" based on nitrogen alone is shown by the broken line with crosses; that based on nitrogen corrected for sodium is shown by the broken line with open circles; and that based on nitrogen corrected for both sodium and potassium is shown by the broken line with open squares. The "caloric discrepancy" curve is omitted. For factors, see Table 6.

For discussion, see text.

was a retention of fluid; since the two curves came together rapidly when the medication was stopped, it is inferred that the extra fluid was quickly lost. Furthermore, since the "caloric discrepancy" curve is the weight curve that the patient would have followed had he remained in nitrogen equilibrium throughout the entire experiment it is of interest that during the first four periods when he was in nitrogen equilibrium, the actual weight curve followed the caloric discrepancy curve.

The third chart (Figure 9) is based on data of a diabetic patient studied by Atchley, Loeb, Richards, Benedict and Driscoll (8) during a period when insulin was withheld.

It will be seen that the "theoretical weight curve" based on nitrogen alone (*i.e.*, not corrected for sodium and potassium) agrees very poorly with the actual weight curve during the period of acidosis resulting from omission of insulin. When the "theoretical weight curve" is corrected for sodium, the agreement is better, but still not good; however, when it is corrected for both sodium and potassium the agreement is excellent. The large correction for potassium (*circa* 2 kg.) is probably too great to be explainable on loss of glycogen alone (*cf.* 390 grams of "wet glycogen" lost by Benedict's fasting man), and hence must represent considerable intracellular dehydration with the acidosis (see whole blood CO_2 curve).

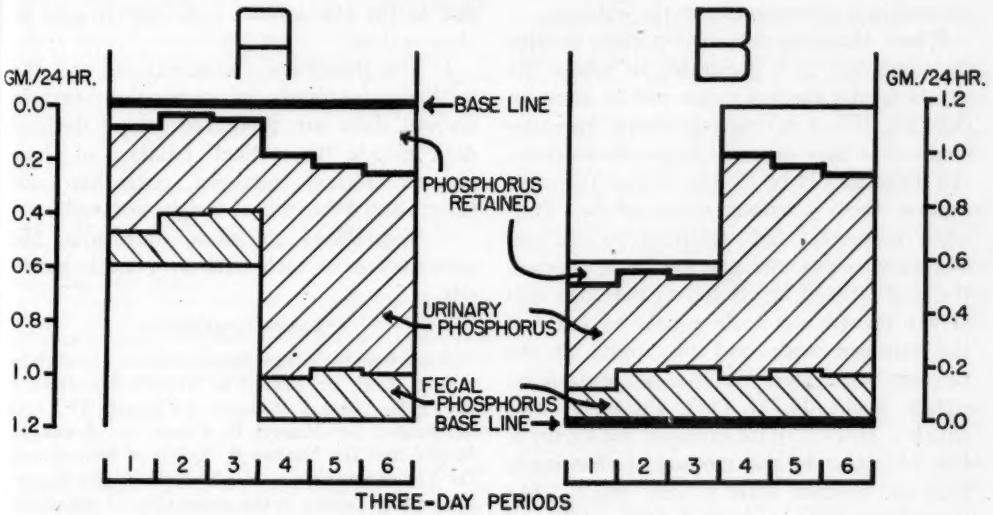

FIG. 10. *Two methods of charting the same metabolic balance data*
A. Method advocated; B. conventional method where intake is plotted from same baseline as excretions. For discussion, see text.

III. PRESENTATION OF DATA

A. *Charts versus tables*

The authors urge that metabolic data be presented in both chart and tabular form (see Figure 2 and Table 5). The former is essential to bring out certain relationships, etc., the latter to allow for recalculation of data if the reader so desires. We agree with Bradford Hill (34) that: "graphs should never be a substitute for statistical tables. An entirely deaf ear should be turned to such editorial pleading as this: 'If we print the graphs would it not be possible to take some of the tables for granted? Having given a sample of the process by which you arrive at the graph is it necessary in each case to reproduce the steps?' The retort to this request is that statistical tables are not a step to a diagram; they are the basic data. Without these basic data the reader cannot adequately consider the validity of the author's deductions, and he cannot do any further analysis of the data, if he should wish, without laboriously and inaccurately endeavoring to translate the diagram back into the statistics from which it was originally constructed."

B. *Suggestions for charting*

After having tried many different kinds of charts, the authors finally selected the one adopted by Bassett (9) as being the most suitable for most types of balance experiments. It includes in the same chart the balance, the intake, and the urinary and fecal excretions. The method of constructing

such a chart is given in the legend to Figure 1. Other examples are given in Figures 5 and 8. The advantage of this method of charting over the more conventional one where the intake and excretion are plotted from the same base line is that it allows one to focus on the most important feature of a balance study, namely the balance (see Figure 10). It will be seen that both methods are equally good in this respect until the intake is changed; this results in the more conventional method in a discontinuity in the balance.

Where the data represent periods greater than one day, it is preferable to reduce the values to the average value per 24 hours in charting. If one is charting several measurements that have constant inter-relationships, the scales should be chosen so that the units express these constant relationships. Thus when metabolic data referring to calcium, phosphorus, and nitrogen are being charted, the units should be chosen so that one unit of the phosphorus scale equals 15 units of the nitrogen scale and two units of the calcium scale [N/P in muscle protoplasm equals about 14.7/1 (13), Ca/P in bone equals 2.23/1 (48)]; for example, see Figure 5. One objection to this method of charting is that the calcium scale is very small; when the calcium data are important an additional chart with a magnified calcium scale may be desirable. Relationships between potassium and nitrogen and sulfur and nitrogen are treated similarly (for ratios see Table 6); for example, see Figure 4.

The authors wish to stress the importance of including base lines, average normal levels and zero points for all data that are charted. It is obviously misleading to chart an alteration in the level of a substance unless the column representing the level begins at zero.

IV. SUMMARY

1. A description is given of the metabolic unit and of the mechanical, chemical, and dietetic methods employed there in the accumulation of data pertaining to metabolic balances, notably those of calcium, phosphorus and nitrogen.

2. The factors involved in the general plan of an experiment, the control observations, the length of "metabolic periods," the selection of a diet, the determination of the contents of a diet by tables and by analysis, the fluid intake, the collection of specimens, and the preparation of specimens for analysis are discussed.

3. A discussion is given of some problems relating to the interpretation of such data, particularly to the detection of gross errors, and to the evaluation of the significance of observations.

4. The theoretical considerations and the methods involved in computing certain derived data are presented; these derived data include "theoretical" balances of phosphorus, calcium, nitrogen, potassium and sulfur, and "theoretical weight curves."

5. Suggestions are made concerning the presentation of such data by graphic methods.

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APPENDIX

A. Methods employed in weighing, cooking, and serving food

All food is weighed on torsion balances, accurate to 0.1 gram; the only exceptions are tea, coffee, and water which are measured in graduated cylinders. To avoid loss in transfer, the food is served as far as possible in the dish in which it was weighed. Food which is to be served hot is usually weighed into individual, highly-glazed pottery casseroles [10.5 cm. (four inches) in diameter, with lids that fit over, not into, the casserole]. Fruits, drained of most of the juice by placing on paper toweling, are weighed into appropriately sized sauce dishes with smooth surfaces. All weighed food to be served hot is heated in a regulation kitchen steamer. The amount of a given food is marked on the casserole lid with a wax pencil which will withstand steam heat, or with nail polish. A dish containing weighed fruit is covered with marked waxed paper secured by a rubber band. A tumbler with weighed fluid is marked either on the glass or by placing a marked cardboard disc called a "butter chip" on the top of the glass and securing it with waxed paper and a rubber band. Weighed salt is never put into food to be heated or cooked, as there are too many opportunities for losses. Food heated in the steamer sometimes gains a small amount of weight by taking on water.

Foods which are not weighed directly into serving dishes include: 1) meat, which is weighed directly into the individual frying pan in which it will be fried or broiled

(see note on the preparation of meat); 2) chicken, raw apples, bananas, cooked potatoes, which are weighed on balanced pieces of waxed paper; 3) sugar, the daily allotment of which is weighed in a glass "double lipped" cream pitcher; 4) spaghetti and uncooked cereals, which are weighed in "dixie cups," and then transferred to bowls for cooking in a measured amount of water; 5) butter, which is weighed on a marked cardboard "butter chip" for each meal; 6) bread, which is weighed on waxed paper in daily amounts rather than for individual meals; 7) salt (C.P. NaCl), which is dried in an electric oven, weighed on a cardboard "butter chip," and then transferred to a marked "coin-envelope" for use throughout the day.

The weighing for the next 24 hours can usually be done in the morning with the exception of a few foods (such as bananas, cooked eggs, *et cetera*) which obviously should be left to the last minute. The bread to be used during a day is weighed at supper time of the preceding day, and is kept well wrapped in large pieces of waxed paper.

In order to have a uniform quality of food available at any time of the year in this section of the country, canned vegetables and fruits are used as much as possible (exceptions: potatoes, carrots, orange juice, lemon juice and orange or grapefruit sections).

B. *Preparation of Food*

1. *Meat:* Lean beef, trimmed of all visible fat, is ground through a "sepra-sieve" to remove the inedible fibers. After the meat has been cooked, the frying pan is scraped clean with the use of a minimal amount of water (not measured) to remove all of the meat juice. This is poured over the cooked meat.

2. *Potatoes:* These are steam-cooked with skins on, cooled, peeled, weighed, and then reheated and served. The use of baked potato is undesirable since it involves the weighing of potatoes before and after baking and the computing of the weight change due to water loss.

3. *Eggs:*

a. *Scrambled:* The beaten whole egg is weighed into a casserole, cooked in a frying pan in part of the patient's butter, transferred to the same casserole and served.

b. *Soft-cooked (boiled):* The egg is cooked the desired length of time, weighed in a sauce dish and served. This method is not wholly desirable in that an egg will seldom weigh the exact amount required. If it weighs less, the difference is made up by incorporating beaten uncooked egg into some item of the dietary; if it weighs more: (a) the excess can be subtracted from an amount of egg to be used later in the day (as eggnog), or (b) parts of yolk and white can be removed.

c. *Fried:* Weigh egg uncooked, cook in part of patient's butter, and put back into dish in which it was weighed. Adjustments for varying weights are made as for boiled eggs.

d. *Poached:* These obviously cannot be used in metabolic studies.

4. *Orange juice:* Strain before weighing.

5. *Recipes:* The use of cooked mixtures is to be avoided, as it entails: 1) too much difficulty in calculations if there are refusals, and 2) an undue amount of work for the dietary staff. Eggnogs of known percentage composition are frequently made, but these do not require the individual computation of water loss that would be required, for example, for custards.

C. *Fluids*

In reporting daily fluid intakes of patients on metabolic regimes no account is taken of the water content of solid foods. The fluid given as water, tea or coffee, milk, cream, and fruit juices is recorded. Small differences due to the specific gravity of liquids are disregarded.

D. *Special Diets*

Most experiments require some special selection of diet which has to be thought through carefully at the beginning of the study. Two aspects will be discussed: a) the low calcium diet, and b) the neutral ash diet.

The details of the low calcium diet were given in the paper by Bauer and Aub (12). This diet requires specially prepared butter (referred to as "butterfat") and "low calcium bread." Recipes for these appear below.

The acid-base value of the ash of the diet is calculated by reducing the cations (Na, K, Ca, Mg) and the anions (PO_4, SO_4, Cl) to millequivalents and balancing the sum of one against the sum of the other. Phosphates are assumed to be divalent in this calculation. By proper selection one can set up a neutral ash diet if desired. However, it is usually easier to adjust the acid-base or the electrolyte content of the ash by adding a suitable weighed salt mixture (*vide supra*).

E. *Serving the Diet*

A dish containing weighed food is considered to be disposed of satisfactorily only when the patient gets from it every possible morsel. As an aid in scraping the dish a small flexible-bladed spatula [blade 2 cm.×12 cm. ($\frac{3}{4}$″×4$\frac{1}{2}$″)] such as is used in a chemical laboratory is given to each patient. This same spatula is used by the dietitian in preparing meat, *et cetera*, before the tray is taken to the patient. Crumbs and bits of food left from one meal may be incorporated into food to be eaten later in the same day.

Finally, the art of dietetics must not be beclouded by a science of dietetics. The success of the experiment depends in no small measure on the rapport which the dietitian establishes and maintains with the patient. In the development of this rapport the ability of the individual dietitian is the essential factor. In the final analysis, the most carefully planned diet is worthless unless the patient will eat it!

F. *Recipes*

1. *Low Calcium Bread*

White flour	435 gm.
Sugar	10 gm.
NaCl (C.P.)	5 gm.
Butterfat	10 gm.
Yeast (compressed)*	6 gm.
Boiling water	260 cc.

* $\frac{1}{2}$ cake of Fleishmann's yeast.

Put butterfat and salt into measured hot water. Let cool to body temperature; add the yeast and sugar, which have been stirred together until liquid; gradually add flour while beating the mixture thoroughly. Turn the partly mixed dough onto a smooth table and knead until all of the flour has been incorporated into the dough. Put the dough back into the same pan to rise until double in bulk, when it may again be kneaded and shaped into a loaf; allow to rise until once again double in bulk; then bake. After the first rising, one can knead the dough, cover well, and leave over night in a refrigerator. A minimal amount of mineral oil is used for greasing the dough and the bread pans. Bake 45 minutes at 375° F.

2. *Butterfat*

Melt one or more pounds of butter over boiling water, pour the melted liquid into a kettle and add about two quarts of boiling water. Cool, place in a refrigerator over night; then knead the hardened fat through successive lots of ice water until the wash water is clear. A fine meshed wire strainer is a convenience in this procedure. Pack the washed butterfat in any suitable covered container.

REFERENCES

1. Albright, F. Cushing's syndrome. Its pathological physiology, its relationship to the adrenogenital syndrome, and its connection with the problem of the reaction of the body to injurious

agents ("alarm reaction" of Selye). *The Harvey Lecture Series 38:* 123–186 (1942–1943).

2. ALBRIGHT, F., W. BAUER and J. C. AUB. Studies of calcium and phosphorus metabolism. VIII. The influence of the thyroid gland and the parathyroid hormone upon the total acid-base metabolism. *J. Clin. Investigation 10:* 187–219 (April 20, 1931).
3. ALBRIGHT, F., W. BAUER, M. ROPES and J. C. AUB. Studies of calcium and phosphorus metabolism. IV. The effect of parathyroid hormone. *J. Clin. Investigation 7:* 139–181 (April, 1929).
4. ALBRIGHT, F., E. BLOOMBERG, and P. H. SMITH. Post-menopausal osteoporosis, *Tr. A. Am. Physicians 55:* 298–305 (1940).
5. ALBRIGHT, F., C. H. BURNETT, O. COPE, and W. PARSON. Acute atrophy of bone (osteoporosis) simulating hyperarathyroidism. *J. Clin. Endocrinol. 1:* 711–716 (September, 1941).
6. ALBRIGHT, F., and R. ELLSWORTH. Studies on the physiology of the parathyroid glands. I. Calcium and phosphorus studies on a case of idiopathic hypoparathyroidism. *J. Clin. Investigation 7:* 183–201 (June 1929).
7. ALBRIGHT, F., W. PARSON, and E. BLOOMBERG. Cushing's syndrome interpreted as hyperadrenocorticism leading to hypergluco-neogenesis; results of treatment with testosterone propionate. *J. Clin. Endocrinol. 1:* 375–384 (May, 1941).
8. ATCHLEY, D. W., R. F. LOEB, D. W. RICHARDS, JR., E. M. BENEDICT, and M. E. DRISCOLL. On diabetic acidosis. A detailed study of electrolyte balances following withdrawal and reestablishment of insulin therapy. *J. Clin. Investigation 12:* 297–326, 1933.
9. BASSETT, S. H. Personal communication.
10. BASSETT, S. H., C. A. ELDEN, and W. S. MCCANN. The mineral exchanges of man. I. Organization of metabolism ward and analytical methods. *J. Nutrition 4:* 235–248 (July, 1931).
11. BASSETT, S. H., and H. E. VAN ALSTINE. Mineral exchanges of man. IV. Variations in the mineral content of diets with a constant raw weight formula. *J. Nutrition 9:* 175–189 (February, 1935).
12. BAUER, W., and J. C. AUB. Studies of inorganic salt metabolism. I. The ward routine and methods. *J. Am. Dietet. A. 3:* 106–115 (September, 1927).
13. BENEDICT, F. G. A study of prolonged fasting. *Carnegie Inst. Wash. Publ. 203:* 1–416 (1915).
14. BODANSKY, A. Phosphatase studies; determination of serum phosphatase. Factors influencing accuracy of determination. *J. Biol. Chem. 101:* 93–104 (June, 1933).
15. BOWES, A. DEP., and C. F. CHURCH. *Food values of proteins commonly used.* Philadelphia, College Offset Press, 1942.
16. BUTLER, A. M., and E. TUTHILL. An application of the uranyl zinc acetate method for determination of sodium in biological material. *J. Biol. Chem. 93:* 171–180 (September, 1931).
17. CALLOW, N. H., R. D. CALLOW, and C. W. EMMENS. Colorimetric determinations of substances containing the grouping —CH CO— in urine extracts as indication of androgen content. *Biochem. J. 32:* 1312–1331 (August, 1938).
18. ELKINTON, J. R. and A. W. WINKLER. Transfers of intracellular potassium in experimental dehydration. *J. Clin. Investigation 23:* 93–101 (January, 1944).
19. EMERY, A. G. and F. G. BENEDICT. The heat of combustion of compounds of physiological importance. *Am. J. Physiol. 28:* 301–307 (September, 1911).
20. FENN, W. O. The deposition of potassium and phosphate with glycogen in rat livers. *J. Biol. Chem. 128:* 297–307 (April, 1939).
21. FENN, W. O. and L. F. HAEGE. The deposition of glycogen with water in the livers of cats. *J. Biol. Chem. 136:* 87–101 (October, 1940).
22. FISKE, C. H. The determination of inorganic sulfate, total sulfate, and total sulfur in urine by the benzidine me hod. *J. Biol. Chem. 47:* 59–68 (June, 1921).
23. FISKE, C. H. and LITARAZEK, in FOLIN, O. *Laboratory manual of biological chemistry.* N. Y. D. Appleton-Century Co. 1934, 5th ed.
24. FISKE, C. H. and M. A. LOGAN. Determination of calcium by alkolimetric titration; precipitation of calcium in the presence of magnesium, phosphate and sulphate, with applications to analysis of urine. *J. Biol. Chem. 94:* 211–226 (September, 1931).
25. FISKE, C. H. and Y. SUBARROW. The colorimetric determination of phosphorus, *J. Biol. Chem. 66:* 375–400 (December, 1925).
26. FOLIN, O. On the determination of creatinine and creatine in urine. *J. Biol. Chem. 17:* 469–473 (1914); Greenwald, I. and J. Gross. The chemistry of Jaffe's reaction for creatinine. A red tautomer of creatinine picrate. *J. Biol. Chem. 59:* 601–612 (April, 1924).
27. FOLIN, O. *Laboratory manual of biological chemistry.* N. Y., D. Appleton-Century Co., 1934, 5th Ed.
28. FOLIN, O. and A. SVEDBERG. Micro methods for determination of non-protein nitrogen, uric acid, and sugar in unlaked blood. *J. Biol. Chem. 88:* 85–96 (August, 1930).
29. FRASER, R. W., A. P. FORBES, F. ALBRIGHT, H. W. SULKOWITCH, and E. C. REIFENSTEIN, JR. Colorimetric assay of 17-ketosteroids in urine. A survey of the use of the test in endocrine investigation, diagnosis, and therapy. *J. Clin. Endocrinol. 1:* 234–256 (March, 1941).
30. GEPHART, F. C. and E. F. DUBOIS. Clinical calorimetry. III. The organization of a small metabolism ward. *Arch. Int. Med. 15:* 829–834 (May, 1915).
31. GUTMAN, A. B. and M. LOW. On the approximation of the calculated to determined calcium content of human dietaries. *J. Nutrition 18:* 257–263 (Sept. 10, 1939).
32. HARRISON, H. E., D. C. DARROW, and H. YANNET. The total electrolyte content of animals and its probable relation to the distribution of body water. *J. Biol. Chem. 113:* 515–529 (March, 1936).
33. HASTINGS, A. B. and L. EICHELBERGER. The exchange of salt and water between muscle and blood. I. The effect of an increase in the total

body water produced by the intravenous injection of isotonic salt solutions. *J. Biol. Chem. 117:* 73–93 (January, 1937).

34. HILL, A. B. *Principles of medical statistics.* London, The Lancet Limited (1939), 2nd Ed., p. 25–26.
35. KENYON, A. T., K. KNOWLTON, I. SANDIFORD, F. C. KOCH, and G. LOTWIN. A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. *Endocrinology 26:* 26–45 (January, 1940).
36. KINSELL, L. W., S. HERTZ, and E. C. REIFENSTEIN, JR. The effect of testosterone compounds upon the nitrogen balance and creatine excretion in patients with thyrotoxicosis. *J. Clin. Investigation 23:* 880–890 (November, 1944).
37. LAVIETES, P. H., L. M. D'ESOPO, and H. E. HARRISON. The water and base balance of the body. *J. Clin. Investigation 14:* 251–265 (March, 1935).
38. LOWRY, O. W. and T. HUNTER. Unpublished adaptation of method by Linderstrøm-Lang, K. and H. Lanz, Jr. *Compt. rend. d. trav. du lab. Carlsberg.* Série Chim. *21:* 315 (1938).
39. REIFENSTEIN, JR., E. C., F. ALBRIGHT, W. PARSON, and E. BLOOMBERG. The effect of estradiol benzoate and of testosterone propionate and of combinations of both on post-menopausal osteoporosis and senile osteoporosis. *Endocrinology 30:* S1024 (1942).
40. ROSE, M. S. *Laboratory handbook for dietetics,* N. Y., Macmillan Co., 1931.
41. ROTH, P. Modifications of apparatus and improved technic adaptable to the Benedict type of respiration apparatus. *Boston M. & S. J. 186:* 457–465, 491–501 (April, 1922).
42. SALTER, W. T., C. FULTON and F. ANGIER. Studies of calcium and phosphorus metabolism. XI. The calculation of acid-base content of the diet. *J. Nutrition 4:* 1–13 (May, 1931).
43. SCHAMBERG, J. F., J. A. KOLMER, A. I. RINGER, and G. U. RAIZISS. Research studies in psoriasis. Second paper. *J. Cutaneous Dis. 31:* 802–915 (November, 1913).
44. SHERMAN, H. C. *Chemistry of food and nutrition.* N. Y., Macmillan Co., 1926, 3rd Ed.
45. SHERMAN, H. C. *Chemistry of food and nutrition.* N. Y., Macmillan Co., 1932, 4th Ed.
46. SHERMAN, H. C. *Chemistry of food and nutrition.* N. Y., Macmillan Co., 1937, 5th Ed.
47. SHERMAN, H. C. *Chemistry of food and nutrition.* N. Y., Macmillan Co., 1941, 6th Ed.
48. SHOHL, A. T. *Mineral metabolism.* N. Y., Reinhold Publishing Corp., 1939, p. 19.
49. SHOHL, A. T. *Mineral metabolism.* N. Y., Reinhold Publishing Corp., 1939, p. 128.
50. TALBOT, N. B., A. M. BUTLER and E. A. MACLACHLAN. The effect of testosterone and allied compounds on the mineral, nitrogen, and carbohydrate metabolism of a girl with Addison's disease. *J. Clin. Investigation 22:* 583–593 (July, 1943).
51. TAYLOR, C. M. *Food values in shares and weights.* N. Y., Macmillan Co., 1943.
52. VAN SLYKE, D. D. and J. M. NEILL. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I., *J. Biol. Chem. 61:* 523–574 (September, 1924).
53. VAN SLYKE, D. D., H. WU and F. C. MACLEAN. Studies of gas and electrolyte equilibria in the blood. V. Factors controlling the electrolytes as well as water distribution in the blood. *J. Biol. Chem. 56:* 765–849 (July, 1923).
54. WALLER, D. S. *Nutritive values of foods.* Revised by George Wahr, Dept. of Dietetics, Univ. of Michigan Hospital, Ann Arbor, Michigan, 1936.
55. WILSON, D. W. and E. G. BALL. Study of estimation of chloride in blood and serum. *J. Biol. Chem. 79:* 221–227 (September, 1928).
56. WILSON, H. E. Relation between sulfur and nitrogen metabolism. *Biochem. J. 19:* 322–337 (1925).
57. WINKLER, A. W., J. R. ELKINTON and A. J. EISENMAN. Comparison of sulfocyanate with radioactive chloride and sodium in the measurement of extracellular fluid. *Am. J. Physiol. 139:* 239–246 (June, 1943).
58. ZIMMERMANN, W. Eine Forbreaktion der Sexualhormone und ihre Anwendung zur quantitativen colorimetrischen Bestimmung. *Ztschr. f. physiol. Chem. 233:* 257–264 (1935); also, Colorimetrische Bestimmung der Keimdrüsehormone. *Ztschr. f. physiol. Chem. 245:* 47–57 (1936).

Pheochromocytoma: Case Report with Certain Observations on the Pathologic Physiology

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HYPERTENSION, paroxysmal or sustained, is a constant feature of pheochromocytomata. In respect to the other clinical and laboratory findings, however, the widest variation is seen. The carbohydrate metabolism and basal metabolic rate in particular are apt to show quite remarkable differences from one case to another. This diversity of response may be explained in several ways (3). The substances secreted by the various tumors may vary slightly in structure and function. The rate of discharge of the hormone into the circulation is certainly variable within wide limits. Finally, the individual tolerance of and adaptive responses to this secretion may determine the final effect. The extent to which these various factors are operative is problematical. Certain observations on the following case may be of some help in the eventual understanding of the mechanisms involved.

CASE REPORT

J.J.H., a 49 year old white male, was first seen as an out-patient on October 9, 1944, and was admitted to the hospital on October 21, 1944. The family history was non-contributory. He gave a history of the usual childhood diseases. From the age of 8 to 16 years he had had recurrent attacks of malaria. At the age of 23 he was struck on the head with the flat surface of a hatchet and rendered unconscious for a short time. There were no other immediate sequelae. At the age of 28 he began having generalized convulsive seizures which recurred every two or three months until the age of 43. During the next five years he was completely symptom-free and enjoyed excellent health. On September 6, 1943, at the age of 48, the patient had another seizure. This attack consisted of a rather unusual group of prodromal symptoms, followed by a convulsion. During the ensuing months the patient noted an increasing number of attacks, most of which did not end in a convulsion. He began to call these his "weak spells." He was never able to tell whether or not a given "weak spell" would end in what he called a "major attack" (generalized convulsion), since the initial sensations were identical. He was unable to relate the onset of the attacks to any cause, including excitement, emotion, hunger, meals, time of day, or posture.

Within a few months the "weak spells" increased in frequency, averaging from two to three attacks daily. On a few occasions there were as many as 10 to 15 attacks in one day. Increasing weakness and nervousness were then noted, and he became aware of almost constant forceful palpitation and heaviness in the cardiac area. Intolerance to heat and increased perspiration became prominent. An increase in appetite and food intake was accompanied by weight loss, amounting to 15 pounds in the year before admission. Nocturia of from one to three times without other urinary symptoms had been present for several months. Fine tremor of the hands was noted, as well as a progressive loss of near vision. There was a pronounced decrease in sexual desire and potency. From September, 1943 to October, 1944, the patient had eight generalized convulsions and innumerable "weak spells." These symptoms were so incapacitating that he had to give up his work eight months before admission.

Social History

The patient was an intelligent, stable, and cooperative individual who had worked as a landscape gardener until the onset of the war, when he took a job as a steamfitter. He had been married 27 years and had five healthy children.

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Physical Examination

The patient was a well developed, fairly well nourished, white male. The pupils were small, but reacted to light and accommodation. The fundi were normal. The heart was normal to percussion and auscultation. Examination of the chest revealed breath sounds of an emphysematous character with some diffuse dry and squeaking râles. The blood pressure on the initial examination was 190 mm. Hg., systolic, and 110 mm. Hg., diastolic, but the patient complained of no symptoms suggestive of a "weak spell." A few hours later the blood pressure was 120 mm. Hg., systolic, and 80 mm. Hg., diastolic. The abdomen was soft and no masses could be palpated. A fine tremor of the extremities was present. The ankle jerks were absent, but no other neurological abnormalities were found.

There was quite striking variation in blood pressure in response to postural changes. In the supine position the pressure was 120 mm. Hg., systolic, and 80 mm. Hg., diastolic. Immediately after standing the blood pressure fell to a systolic level of 70 or 80 mm. Hg. Within a few minutes it rose to 170 or 180 mm. Hg., systolic, only to fall to normal shortly thereafter.

Description of Attacks

Following routine hyperventilation for electroencephalography the patient complained of having a "weak spell" and it was found that he would invariably have an attack after 15 to 20 deep inspirations. Observation of self-induced attacks, plus the patient's descriptions of the same, yielded the following information: An initial feeling of weakness and mild nervousness was followed by a rising sensation in the epigastrium and nausea without vomiting. Marked palpitation and a throbbing sensation in the head, observed by the patient to be synchronous with the heart's beat, was noted next, accompanied by a sensation of pressure on the sides of the head with moderate headache. A coarse tremor as well as numbness and tingling of the hands and arms was present throughout the seizure. Early in the attack there was extreme blanching of the face and upper extremities and a marked degree of "goose-flesh." The extremities became cold and slightly moist. The radial pulse became slower and much reduced in amplitude. In the latter part of the attack the pallor gave way to very marked flushing, and the patient became drenched with perspiration. After about three or four minutes the acute symptoms subsided,

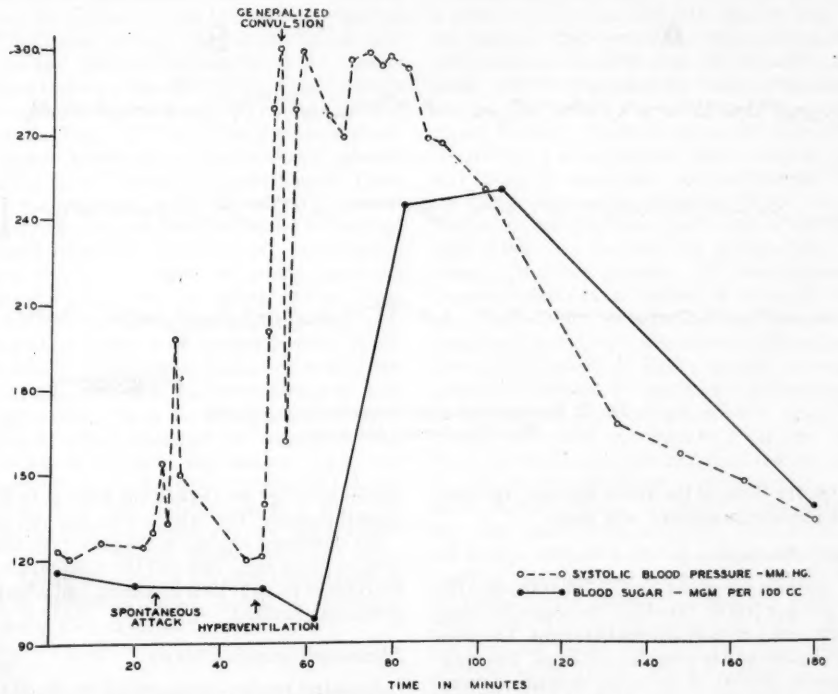

FIG. 1. Blood pressure and blood sugar changes during a hypertensive paroxysm.

leaving the patient weak and "washed-out." During the typical spell there was no loss of consciousness. On one occasion, however, a rather severe induced paroxysm ended in a generalized convulsion. The blood pressure and blood sugar changes during an attack are shown in Fig. 1. The skin temperature fell 1 to 2° C. in the blanching phase, and became slightly higher than normal in the period of flushing. Abdominal massage, changes in posture and carotid sinus pressure failed to precipitate an attack. No significant predisposing factor for the numerous spontaneous seizures which occurred during his hospital stay was found. On the basis of the above findings, the diagnosis of pheochromocytoma was made.

Oct. 29, 1944						
Blood sugar	119	215	107	71	91	91
Sugar in Urine	0	3+	2+	2+	2+	trace

The basal metabolic rate on two occasions was +4% and −2%. A gastro-intestinal x-ray series showed no abnormalities. The spinal fluid was normal. A pneumoencephalogram was interpreted as being within normal limits. An orthodiagram showed the heart to be normal in size and shape, but a moderate degree of uncoiling and dilatation of the thoracic aorta was observed. A roentgenogram of the abdomen did not reveal any displacement of the kidney shadows. In

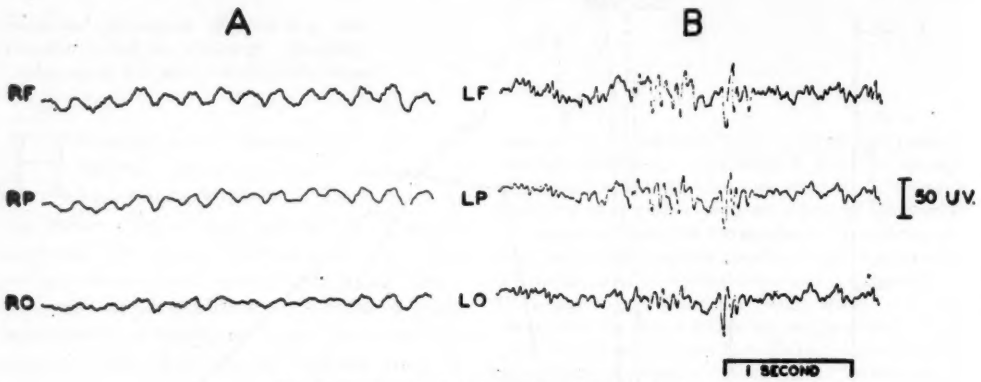

FIG. 2. Electroencephalogram before (A) and during (B) a hypertensive paroxysm.

Laboratory Findings

The erythrocyte count was 5,200,000; the Hb. was 13 gm. per 100 cc. (Haden); the leucocyte count was 9,400 with a normal differential count. The urine showed occasional fine granular cell casts. The maximum specific gravity of the urine on routine examination was 1.024. An occasional fasting specimen of urine showed two plus or three plus sugar. The urea clearance was 60%; the value for the PSP test was 85% in two hours. The blood urea was 34 mg. per 100 cc. The blood Wassermann and Kahn reactions were negative. Blood chlorides were 580 mg. per 100 cc. The blood cholesterol was 243 mg. per 100 cc. with 30 per cent in the esterified form. The fasting blood sugar values on several occasions were 163, 114, 104, 119, 152, and 142 mg. per 100 cc. respectively. Five-hour glucose tolerance tests (100 gm. glucose given orally; determinations made on venous blood) on Oct. 11, 1944 and on Oct. 29, 1944, are shown in the table below:

Oct. 11, 1944						
Hour	Fasting	½	1	2	3	4	5
Blood sugar	163	253	248	112	74	90	105
Sugar in Urine	0	3+	2+	2+	2+	2+	trace

travenous urograms showed the kidneys to be in the normal position. The calyces were not well outlined.

No significant change in the pulse rate, blood pressure, or blood sugar followed the subcutaneous injection of 0.75 cc. of 1:1000 adrenalin, and no subjective symptoms resulted.

Electrocardiographic Changes

A control tracing during which the blood pressure was 140 mm. Hg., systolic, and 90 mm. Hg., diastolic, with a rate of 107 per minute, was interpreted as being within the limits of normal variation. During a typical seizure precipitated by hyperventilation, the blood pressure rose to 300 mm. Hg., systolic, and 180 mm. Hg., diastolic. A tracing at this time showed a change in rhythm characterized by a slowing of the ventricular rate to 75 per minute and alterations of the relation of the P waves to the ventricular complexes. The P wave changed from the previous normal upright position to an inverted form partially buried in the S wave. However, the inverted P wave was only visible in every other QRS complex. These changes were interpreted[1] as probably representing a shift of the pacemaker to the central part of the AV node with varying retrograde conduction. The trac-

[1] By Dr. Frank N. Wilson, of Ann Arbor, Mich.

ing revealed a predominant 2:1 retrograde block of the impulse from the AV node to the auricle during the height of the attack. This was followed by a period when there was a lessening of the degree of block where more impulses were transmitted to the auricles with a progressive prolongation of the conduction time (R-P interval) suggesting a retrograde Wenckebach phenomenon.

Electroencephalograms[2]

Using a three lead Grass electroencephalograph, a tracing was taken on October 10, 1944. With leads attached to the right frontal, parietal, and occipital areas, and the patient at rest, the tracing in general showed more slow activity than in the normal person of this age. Scattered through all of this portion of the record were numerous 6 to 8 per second, smooth rounded waves of approximately 20 to 40 microvolts in amplitude (Fig. 2A). In addition, there were short, 2 to 3 second bursts of 5 to 6 per second, smooth waves rising up to 75 microvolts in amplitude. These were most prominent in the frontal and parietal leads. There was no difference between the tracings as recorded from the right and left hemispheres. After hyperventilation there was no change in the above type of waves, but low voltage, 15 to 25 per second waves were superimposed on the basic pattern. In addition there were numerous short bursts of 15 to 25 per second waves, beginning at approximately 20 microvolts but rapidly reaching as high as 100 microvolts (Fig. 2 B). During each effort at hyperpnea an attack occurred but was not related to any constant change in the tracing, other than the introduction of the fast activity described above.

A second EEG was obtained on Oct. 29, 1944. This tracing showed less slow activity and featured a more prominent, though slightly irregular, alpha rhythm. Again, however, a hypertensive paroxysm induced by hyperventilation caused no essential change in the EEG tracing.

Operation[3]

Supplemental doses of sodium chloride were given for several days pre-operatively. The patient was given 10 cc. of adrenal cortex extract (Upjohn) and 5 mg. desoxycorticosterone acetate (Percorten-Ciba) intramuscularly on the night prior to the operation, and again just prior to surgery. Basal anesthesia with avertin was supplemented by nitrous oxide-oxygen-ether. A midline abdominal approach was used, since it was considered best to explore both adrenals.

The left kidney and adrenal appeared normal to palpation. Above the right kidney was a fixed cystic mass about the size of an orange. It was attached high up under the diaphragm and moved with each respiration. The incision was extended, in the form of a T, to the right anterior axillary line, and this afforded adequate exposure. The hyperpnea incident to induction of anesthesia caused a moderate rise in the systolic blood pressure, but palpation of the tumor caused extreme rises up to and more than 250 mm. Hg., systolic. The tumor was covered by very friable veins, and bleeding of about 500 cc. resulted during its removal.

Immediately after removal of the tumor the systolic blood pressure dropped to *ca.* 60 or 70 mm. Hg. It was necessary to give intravenously small doses of adrenalin to counteract this drop, but it seemed impossible to stabilize the pressure. After 0.25 to 0.5 cc. of intravenous adrenalin, the pressure would rise to a systolic level of *ca.* 130 mm. Hg. for from five to ten minutes. There would then be a moderately rapid drop to about 50 or 60 mm. Hg., systolic, and this could only be controlled by more intravenous adrenalin. The wound was closed and 5 cc. of adrenal cortex extract (Upjohn) was given intravenously. In addition 5 cc. of adrenal cortex extract (Upjohn) and 10 mg. of desoxycorticosterone acetate (Percorten-Ciba) were given intramuscularly. The blood loss was replaced by two transfusions of whole blood, each of 500 cc., but this was of little help in controlling the blood pressure. The subcutaneous and intramuscular administration of adrenalin had no appreciable effect. With each drop of the pressure to dangerous levels, the respirations diminished and cyanosis appeared. It finally became necessary to give the adrenalin in continuous intravenous drip at the rate of 32 drops per minute, using a 1:20 dilution of 1:1000 adrenalin in 5 per cent glucose. The pulse was regular but remained fast throughout, *ca.* 130 per minute. After seven hours the systolic blood pressure became stabilized at about 130 mm. Hg., but would immediately fall when the drip was cut down to from 20 to 24 drops per minute. The blood sugar at this time was 300 mg. per 100 cc. Ten cc. of adrenal cortex extract (Upjohn) was given intramuscularly. The patient remained fairly stable for the next ten hours, but then the pulse and blood pressure failed badly. He was revived by large amounts of intravenous and intracardiac adrenalin. However, within three hours the blood pressure failed to respond further to intravenous adrenalin, and he died about 20 hours post-operatively.

Pathological Examination

The tumor consisted of an ovoid mass measuring 8×7×5 cm. It was composed of solid, pinkish-yellow tissue which enclosed a large cyst filled with bloody fluid. The cyst's wall varied from 0.5 cm. to 3 cm. in thickness. Histologically the tissue was encapsulated and showed tumor cells growing in strands and masses as well as diffusely. The cells were generally polygonal in shape with smooth cytoplasm and eccentrically placed nuclei, which in some places were dark and in others were vesiculated with prominent nucleoli. The cells varied in size and shape

[2] The EEG tracings were interpreted by Dr. Hugh Page Newbill.

[3] The operation was performed by Dr. Samuel A. Vest.

and there was a tendency to form giant cells, some of which contained seven or eight nuclei. The cells also formed whorls and small bundles resembling neural tissue. The histological diagnosis was pheochromocytoma.

An autopsy was performed and was entirely negative. The left adrenal was normal. The heart, brain, and kidneys were within normal limits and showed no significant vascular disease. The pancreas was normal. There had been no post-operative hemorrhage.

STUDIES OF THE TUMOR FLUID AND TISSUE GLYCOGEN[4]

Biological assay of the fluid from the cystic tumor and an estimation of the liver, heart, and muscle glycogen after death were carried out.

The assay was done by the intravenous injection of the diluted fluid into dogs under light amytal anesthesia, with direct blood pressure readings by means of a carotid cannula. The tests were made six hours post-operatively, the fluid being kept at 3° C. during the interval. The fluid was found to contain a considerable quantity of pressor substance. The blood pressure response to 0.05 cc. of the tumor fluid was equivalent to that observed after the intravenous injection of 0.25 cc. of a 1:100,000 solution of adrenalin. The fluid was kept at 3° C. for 48 hours, and then was re-tested in the same fashion. It was surprising that the pressor substance was still present in undiminished strength, and compared to similar quantities of adrenalin. In both titrations the blood pressure curves following the injection of the tumor fluid were indistinguishable from those following adrenalin.

The autopsy was performed six hours following death. Samples of striated muscle, liver, and heart muscle were taken and the glycogen content of each determined. All of the values were very low, but the heart muscle was particularly depleted of glycogen.

DISCUSSION

This case is unusual in that generalized convulsions were an important part of the symptomatology. It was impossible to obtain a clear-cut history of the paroxysmal attacks in association with the first series of convulsions from the age of from 28 to 43. The first unequivocal description of the hypertensive attacks was dated September, 1943. The electroencephalogram neither confirmed nor eliminated the possibility of post-traumatic or idiopathic epilepsy. It was assumed that the paroxysms of hypertension merely furnished the exciting stimulus in a person with a low convulsive threshold. The presence of pilomotor phenomena is another unusual finding (6).

The induction of attacks by hyperventilation was an accidental discovery, but proved uniformly successful. As noted previously the tumor was fixed very close to the diaphragm and moved downward with each inspiration. Pressure on the tumor during the excursions of the diaphragm may thus have been responsible for the paroxysms following hyperventilation (7). A possible mechanism for this method of inducing attacks suggests itself in connection with certain features of this case. The concentration of adrenalin in adrenal-vein blood has been estimated by various workers and methods, and the figures quoted in the literature vary from 1:360,000 to 1:1,000,000,000. The pressor equivalent of the present tumor fluid—1:500,000—would therefore be similar to the highest concentrations found in the body. Since the tumor sac contained about 150 cc. of free fluid, it is conceivable that large amounts of an extremely potent "hyperpietic factor" might have been liberated into the general circulation of the patient under certain provocative conditions such as hyperventilation.

Electrocardiographic changes during attacks are usually not very striking. Burgess, Waterman, and Cutts (2) reported a case in which the AV node showed increased irritability during the paroxysms. The findings in the present case are possibly due to a strong compensatory vagal response to the extremely high blood pressure.

The tendency toward hyperglycemia and glycosuria is interesting in view of the finding of diabetes mellitus due to pheochromocytoma by Howard and associates (3). In

[4] These determinations were made by Dr. S. W. Britton.

the present case, however, the response to hyperglycemia was unimpaired, as manifested by the rapid fall in blood sugar during the glucose tolerance tests.

In the reported case localization of the tumor was not definitely established prior to the operation. Peri-renal air insufflation was not done for the following reasons: The procedure has resulted in some serious reactions, and does not rule out a tumor even when negative. The position of the kidneys on roentgenographic examination was normal. The clinical picture was so distinctive that exploration was definitely indicated. It was thought that palpation of both adrenals at operation was a desirable precaution. In retrospect, however, the surgeon was of the opinion that if the tumor had been localized, a posterior approach in this case would have made removal somewhat easier. For this reason, pre-operative localization of the growth would be highly desirable and perirenal air injection would seem to be of value if other methods give no definite information.

A large proportion of cases have a profound drop in blood pressure as soon as the blood vessels from the tumor are ligated. The resulting "shock" is remarkable in that it responds readily to adrenalin and occasionally only to adrenalin. In the present case the absolute dependence of the blood pressure on the intravenous administration of adrenalin necessitated the use of large amounts of this substance. At no time did the patient seem able to maintain an adequate blood pressure without the aid of adrenalin. It seems unlikely that cortical deficiency could have been an important factor since the left adrenal was normal, and also in view of the fact that liberal amounts of cortical extract and desoxycorticosterone were given. Death apparently resulted from cardiac exhaustion. The low glycogen content of the heart muscle lends support to this view, although postmortem changes may have been partially responsible for the glycogen loss.

It is quite generally assumed that the clinical manifestations depend on the paroxysmal release of large quantities of adrenalin into the general circulation. The presence of a pressor substance in the blood during attacks has been shown by biological methods (1). Using a chemical method, Strombeck and Hedberg (12) reported finding 1000 times the normal adrenalin content in the blood during a paroxysm. In the interval between attacks they found 30 times the normal amount. Their determination depended on decolorization of methylene blue and is of questionable accuracy. We attempted to estimate the adrenalin content of the blood during an attack by the method of Raab (9) but were unsuccessful due to technical difficulties. In this regard the finding of an increased serum potassium level during attacks by Strickler (11) and between attacks by MacKeith (8) is of interest, since the intravenous injection of adrenalin in man results in a momentary elevation of the serum potassium, followed by a slight fall (5).

However, it seems apparent that the paroxysmal release of adrenalin does not account for all the findings. The cited case manifested constantly a fast pulse, increased perspiration, intolerance to heat, high fasting blood sugar, occasional glycosuria, weight loss with ravenous appetite, and normal basal metabolic rate. Another reported case (4) had a continuously elevated basal metabolic rate and reduced glucose tolerance, both returning to normal after removal of the tumor. Also, the patient with sustained hypertension usually has a normal blood pressure after operation (10). All these considerations support the suggestion of Evans (4) that there is a constant hypersecretion of adrenalin in some cases. This might also explain the profound post-operative fall in blood pressure. Apparently certain patients become abnormally dependent on adrenalin to maintain their normal blood pressure. Success of the operation seems to rest partly on the ability of the patient to mobilize the normal pressor mechanisms after this abnormal stimulus is removed.

It is noteworthy that the pressor substance in the tumor fluid did not diminish in strength over a period of 48 hours. Hence it is unlikely that adrenalin was present unless in some un-

usually stable form. A relatively stable "mother substance" (tyrosine?) rather than adrenalin itself may have been involved in this case. This finding supports the possibility that the active principle secreted by pheochromocytomata may not be identical in all cases (3). Substantiation of this important concept is not available at present.

SUMMARY

A case of pheochromocytoma of the adrenal medulla is described. The clinical picture was typical of this condition. In addition, the patient suffered from generalized convulsions apparently precipitated by the hypertensive paroxysms.

Surgical removal of the tumor was followed by intractable shock and death.

Certain observations on the mechanisms involved in this symptom-formation are presented.

(We wish to thank Dr. David C. Wilson for permission to report this case.)

REFERENCES

1. BEER, E., F. KING, and M. PRINZMETAL. Pheochromocytoma with demonstration of a pressor substance in the blood. *Ann. Surg. 106* (1): 85–91 (1937).
2. BURGESS, A. M., G. W. WATERMAN, and F. B. CUTTS. Adrenal sympathetic syndrome with unusual variation in cardiac rhythm; report of case. *Arch. Int. Med. 58* (3): 433–447 (1936).
3. DUNCAN, JR., L. E., J. H. SEMANS, and J. E. HOWARD. Adrenal medullary tumor (pheochromocytoma) and diabetes mellitus; disappearance of diabetes after removal of the tumor. *Ann. Int. Med. 20* (5): 815–821 (1944).
4. EVANS, W. F., and H. J. STEWART. The peripheral blood flow in a case of adrenal pheochromoctyoma before and after operation. *Am. Heart J. 24* (6): 835–842 (1942).
5. FENN, W. O. The role of potassium in physiological processes. *Physiol. Rev. 20* (3): 377–415 (1940).
6. HOWARD, J. E., and W. H. BARKER. Paroxysmal hypertension and other clinical manifestations associated with benign chromaffin cell tumors (phaeochromocytomata). *Bull. Johns Hopkins Hosp. 61* (6): 371–410 (1937).
7. HYMAN, A., and W. H. MENCHER. Pheochromocytoma of the adrenal gland. *J. Urol. 49* (6): 755–771 (1943).
8. MACKEITH, R. Adrenal-sympathetic syndrome. *Brit. Heart J. 6* (1): 1–12 (1944).
9. RAAB, W. Pathogenic significance of adrenalin and related substances in the heart muscle. *Exper. Med. & Surg. 1* (2): 188–225 (1943).
10. SMITHWICK, R. H. Some experiences with the surgical treatment of hypertension in man. *Tr. & Stud., Coll. of Physicians, Philadelphia. 12* (3): 93–100 (1944).
11. STRICKLER, JR., C. W. Pheochromocytoma; operative failure; case report. *South. Surgeon 11* (3): 193–199(1942).
12. STROMBECK, J. P., and T. P. HEDBERG. Tumor of the suprarenal medulla associated with paroxysmal hypertension. *Acta Chir. Scandinav. 82* (2–3): 177–189 (1939).

Effect of Thiouracil on Human Tissues

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OPPORTUNITY to observe the effect of thiouracil on human tissues was offered recently by the sudden death from cerebellar hemorrhage of a patient suffering from thyrotoxicosis which had been successfully controlled over a period of 381 days by the continuous administration of thiouracil.

CASE REPORT

A white, unmarried woman, aged 74, consulted the writer on May 5, 1944 because of shortness of breath, palpitation, nervousness, sweating and weight loss of 15.9 kg. (35 lbs.) during the preceding year. She had had a goiter for many years, but it had never been found to be hyperactive. Elevated blood pressure values had been obtained for at least 15 years; they were discovered when she had a stroke, causing a left sided paralysis from which she had made satisfactory recovery. She had been taking iodine for four weeks prior to her first visit.

In appearance she was lethargic in movement, doddering and emaciated, but had an unusually bright eye, and an alert mentality. Lid-lag was present. There was a coarse intention tremor overshadowing a fine tremor of both hands. The palms were warm and moist, the pulse rate, 116, and the blood pressure, 190/110. Both lobes of the thyroid were enlarged and firm, and there was an irregular adenoma measuring about 3 cm. in diameter in each lobe. The tongue was beefy red and swollen, and the corners of the mouth were cracked. The heart was enlarged toward the left, the point of maximum intensity being 11 cm. from the midsternal line in the fifth left interspace; the aortic second sound was markedly accentuated and there was a roughening of the first sound which was loudest along the left sternal border. Both feet were cold and cyanotic, and the dorsalis pedis pulsation was absent. The deep reflexes on the left side were hyperactive. The basal metabolic rate was plus 38 per cent, and the blood cholesterol 176.4 mg. per cent. All other laboratory studies were within normal limits.

Thiouracil was started at the rate of 0.2 gm. given four times daily, and large doses of vitamin concentrates were administered. Clinical improvement was noted by the patient within four weeks. She had gained nearly one kilogram (2 lbs.), and was less tired and nervous, although the basal metabolic rate at this time was plus 40 per cent and the blood pressure was 200/120 mm. Hg. Thiouracil in doses of 0.8 gm. was continued daily for 109 days, at the end of which time the basal metabolic rate was plus 1 per cent, the pulse rate, 78, and the blood pressure, 170/100. The thyroid enlargement had decreased, and the gland was softer in consistency. The dosage of thiouracil was gradually reduced as follows: 0.6 gm. daily for 38 days; 0.4 gm. daily for 21 days; 0.2 gm. daily for 31 days; 0.3 gm. daily for 170 days; and 0.2 gm. daily for 12 days. The patient received a total of 178 gm. in 381 days.

Clinical improvement was quite remarkable. The pulse rate now ranged between 68 and 78 per min., the blood pressure leveled off at 160/100 mm. Hg., and the nervousness, fine tremor and lid-lag were gone. The basal metabolic rate ranged between plus 1 per cent and minus 7 per cent during the last nine months of treatment and there was a total weight gain of 5.7 kg. (12½ lbs.).

On May 21, 1945 she awoke at eight o'clock in the morning, but returned to bed shortly because of severe dizziness and headache. Vomiting set in, and by 10:00 A.M. she had lost consciousness and developed a flaccid paralysis of all limbs. Cyanosis developed gradually, and she expired at 3:30 P.M. Autopsy was performed about three hours later.

Autopsy

The body was that of a well developed, well nourished elderly white female 64 in. tall and weighing 135 lbs. There was no gas or free fluid in the peritoneal cavity and the abdominal organs were normally situated. After the chest plate was removed the thyroid was completely dissected out. It weighed 55 grams, was slightly enlarged, reddish-purple in color, and nodular in appearance. On cut section it was slightly fibrous with multiple adenomata scattered throughout. One nodule, white in color, was firmly encapsulated in the left upper pole. No calcification was noted.

The heart weighed 375 grams. The left ventricular wall was slightly thickened and was dark red in

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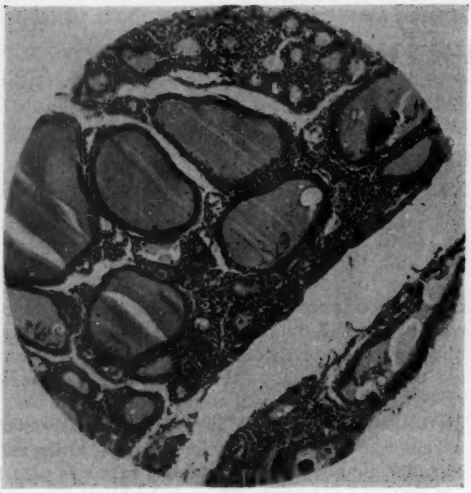

FIG. 1. Thyroid showing diffuse hyperplasia and terminal colloid retention (×150).

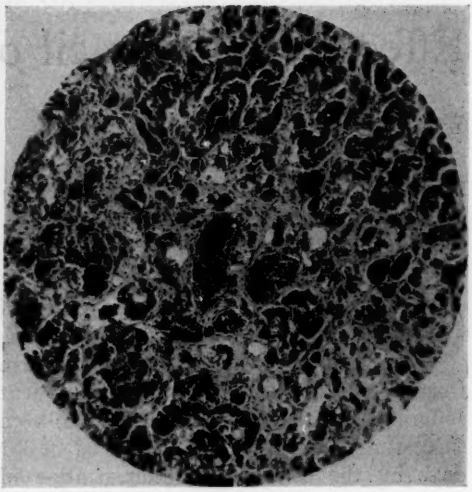

FIG. 3. Anterior pituitary showing depression of eosinophilic elements (×150).

color. The aorta showed some calcification. The coronary arteries showed thickened walls with some calcification. No significant valvular changes were noted. The right lung weighed 550 gm. and the left 450 gm. Both lower lobes showed hypostatic congestion. The liver weighed 1160 gm., was reddish-brown in color, and there was no evidence of increased fibrosis. On cut section it showed only moderate congestion with scattered yellow mottling. The spleen weighed 175 gm., and its appearance was not remarkable. The pancreas, weighing 90 gm., was quite normal in appearance. The right kidney weighed 125 gm. and the left 130 gm. Noteworthy changes were absent. Both adrenals were somewhat larger than normal, with a nodular surface, the nodules being bright yellow in color. There was mild autolysis of the medulla.

The brain showed only moderate edema on its external surface. After fixation and sectioning, a cavity 5 cm. in diameter filled with clotted blood was found in the left cerebellum. The hemorrhage had extended into the third and fourth ventricles.

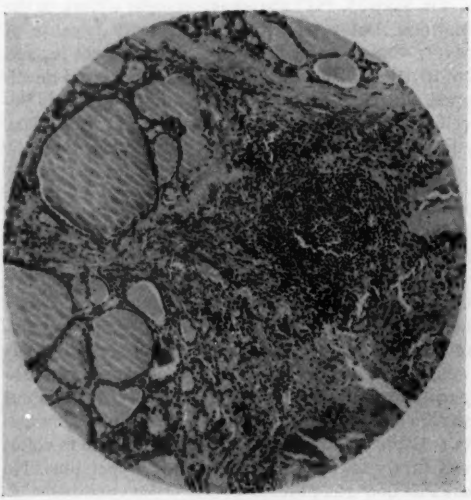

FIG. 2. Thyroid with lymphoid area (×150).

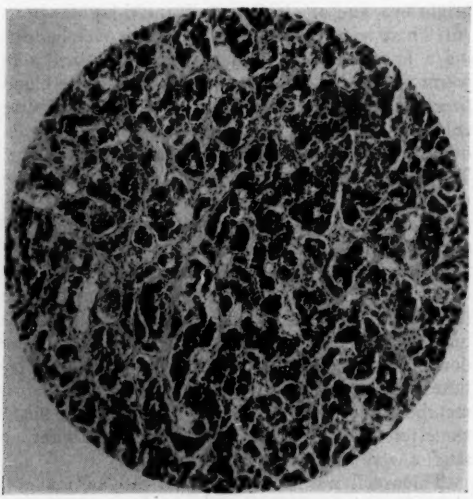

FIG. 4. Anterior pituitary showing basophilic hyperplasia (×150).

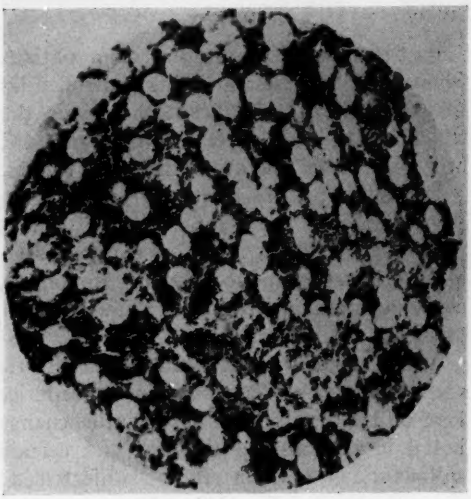

FIG. 5. Bone marrow hyperplasia (150).

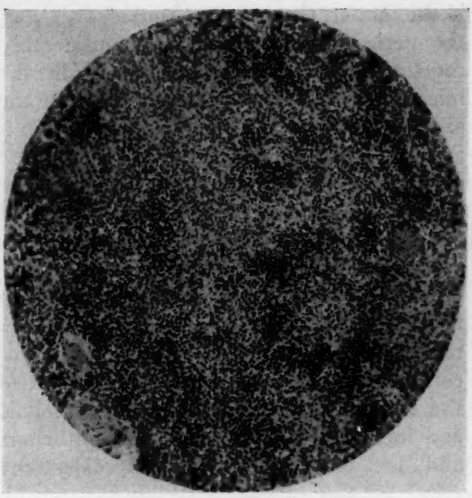

FIG. 6. Spleen showing pulp hyperplasia with beginning hemopoesis (150).

The pituitary gland weighed 75 gm. and measured 1×1.5×1 cm. It was slightly enlarged and showed the impress of its surrounding bony structure.

Microscopic study of the thyroid gland showed diffuse hyperplasia with terminal colloid retention. There were many lymphoid areas and some papillary excrescences of the follicles. (Fig. 1 and Fig. 2).

The pituitary showed early atrophic changes and a disappearance of the eosinophilic elements was noted (Fig. 3). The pars intermedia and pars nervosa appeared normal. The anterior lobe was enlarged, due mainly to increased fibrous tissue and dilatation of the blood spaces, but there was increased basophilia especially around the outer margins (Fig. 4).

The bone marrow was hyperplastic for an individual of this age (Fig. 5).

The suprarenal gland showed an increase in the columnar portion of the cortex with marked lipoidosis. The glomerular zone was a thin sheet beneath the capsule. The spleen showed hyperplasia of the pulp and islands of active hemopoiesis (Fig. 6).

Miliary hemorrhages were found in the pons and cerebellum.

DISCUSSION

In reviewing the literature, the only comparable instances of extensive post mortem studies made on thiouracil-treated patients are those of Kahn and Stock (2) and Ferrer, Spain and Cathcart (1). In the first instance the patient was a diabetic woman, aged 62, who developed fatal agranulocytosis after 30.8 gms. of the drug had been administered over a period of 51 days. There was jaundice, and the liver was slightly smaller and softer than average. The pancreas was much smaller than average and appeared atrophic both grossly and microscopically. The adrenal glands showed moderate atrophy of the cortical tissue, and pronounced vascular engorgement. The thyroid gland was diffusely hyperplastic, considerably infiltrated by lymphocytes and contained a few small areas of fibrosis. The pituitary and brain presented no abnormality on gross or microscopic examination.

The second study was that of a 70 year old male dying of agranulocytosis after administration of 113.6 gm. of thiouracil during 128 days. Autopsy was performed 48 hours after death. The right lobe of the thyroid (which was most involved) showed numerous adenomas; for the most part the follicles were filled with colloid and were lined with low cuboidal cells. An occasional colloid-free focus showed more hyperplastic lining cells. Considerable fibrosis was present. The bone marrow showed moderate hypoplasia of the granulocytic cells with decreased percentage of polymorphonuclear cells.

A fair comparison of the findings in the above mentioned cases with those in the patient reported here can not be made because death was attributable to thiouracil,

in the former, there was agranulocytosis, and the period of treatment was considerably shorter. Little parallelism is noted in the microscopic changes in the tissues with the exception of those in the thyroid. No pituitary gland changes were observed in the first case report, (2), while in the present case the changes were significant and simulated closely those noted in experimental studies in animals.

In this latter respect, the changes noted by the MacKenzies (3) in animal tissues after the use of certain sulfonamides and thiourea consisted of hyperemia and enlargement of the thyroid gland with reduction in colloid and increase in height of acinar epithelium and the development of "thyroidectomy cells" in the pituitary. Williams and his co-workers (4) on the other hand, failed to find any alteration in the structure of the pituitary of animals fed thiouracil for intervals of from 7 to 60 days. Nor was there any observed effect on the adrenals or gonads.

It is apparent that little human material is at present available with which the findings reported here may be compared. The diffuse hyperplasia in the thyroid has been a generally observed thiouracil effect but its finding here, combined with terminal colloid retention, indicates an impending exhaustion of the thyroid. The present observations, particularly the changes in the pituitary, though limited to only one instance, appear substantially representative of thiouracil effect on human tissues where no toxic effect has been produced by the drug.

SUMMARY

Post-mortem tissue studies on a patient whose thyrotoxicosis was being controlled by thiouracil showed significant changes in the thyroid, pituitary, spleen and bone marrow, but very little alteration in other tissues.

Diffuse hyperplasia was present in the thyroid. There was also terminal colloid retention which might be interpreted as an early exhaustion induced by the prolonged, continuous administration of thiouracil (381 days).

The disappearance of eosinophilic elements and the increased basophilia in the anterior lobe of the pituitary simulated the change noted in animals fed thiourea and certain sulfonamides and in those subjected to thyroidectomy, where the development of so-called "thyroidectomy cells" takes place.

Hyperplasia of the bone marrow, and the splenic pulp, with evidence of active hemopoiesis, could well have resulted from the increased pituitary basophilia.

Thanks is due Dr. Plinn F. Morse for his study of tissues.

REFERENCES

1. Ferrer, M. Irene, D. M. Spain and R. T. Cathcart. Fatal agranulocytosis resulting from thiouracil. *J.A.M.A. 127:* 646 (Mar. 17, 1945).
2. Kahn, Julius and R. P. Stock. Fatal agranulocytosis resulting from thiouracil. *J.A.M.A.126:* 358 (Oct. 7, 1944).
3. MacKenzie, C. G. and Julia B. MacKenzie. Sulfonamides, thioureas and thyroid. *Endocrinology 32:* 185 (Feb. 1943).
4. Williams, R. H., A. R. Weinglass, G. W. Bissell and Jean B. Peters. Anatomical effects of thiouracil. *Endocrinology 34:* 317 (May 1944).

ASSOCIATION NOTICE

THE Government's lifting of the ban on conventions permits the holding of the 1946 scientific assembly of The Association for the Study of Internal Secretions.

Pursuant to the vote of the members at the last business session, this meeting will be held in the same city and on approximately the date of the meeting of the American Medical Association; however, this may be changed at the discretion of the Council. Time and place will be announced later.

Members or others (introduced by members) desiring to present papers should send four copies of an abstract of the work they desire to present to Dr. Carl R. Moore, Department of Zoology, University of Chicago, Chicago 37, Illinois, before April 1, 1946.